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ABSTRACT

This book contains 22 lessons using hands-on activities designed to present some aspect of biotechnology in a usable form that teachers can adapt for their classrooms. The introductory section serves as a resource that introduces the teacher and student to the history of biotechnology. The activities are divided into five units that group lessons by concept. Lessons from the five units are designed to: (1) teach the basics of DNA and heredity; (2) focus on the omnipresence of DNA and show students that it can be extracted from any organism and analyzed; (3) illustrate the way that DNA is naturally manipulated by organisms; (4) focus on how humans can manipulate DNA to achieve particular objectives, using examples from DNA fingerprinting, soil organisms, and cancer; and (5) address the ethical questions that biotechnology has raised and involve students in decision-making exercises. Each lesson consists of teacher and student pages that contain background information, preparation procedures, lists of materials, answers to student questions, and additional resources. Appendices contain a glossary of 46 biotechnology terms and a list of 170 general resources. Resources include books and journal articles listed by the following topics: agriculture, aquaculture, biotechnology education, DNA fingerprinting, environmental release, ethics, medicine, patents, public understanding, and waste disposal. (MDH)

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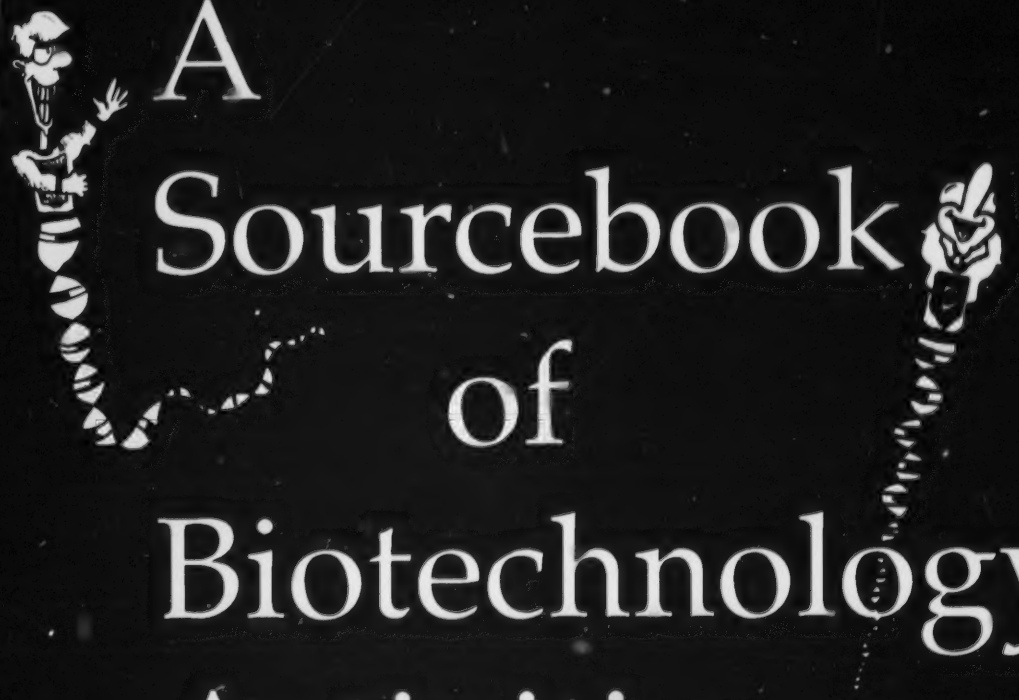

A Sourcebook of Biotechnology Activities

National Association of Biology Teachers
North Carolina Biotechnology Center

Alison M. Rasmussen
•
Robert H. Matheson III
Project Co-directors

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A Sourcebook of Biotechnology Activities

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National Association of Biology Teachers
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About This Book

Why Biotechnology?

One of the keys to an exciting science class is relevance. Teaching students something that applies directly to everyday life, or that is discussed regularly in the media is a surefire way to capture their attention. The challenge for teachers has always been to take current scientific discoveries and technology and present them to students within the confines of a typical school schedule and classroom. Teachers with years of experience often become quite good at translating new, complex information into simple, easy-to-prepare activities, but even a master teacher cannot spare the time or energy to devise new laboratories on a regular basis. This book represents the combined brain power of many experienced teachers who wished to share their "translations" of biotechnology with their colleagues. Each lesson is designed to present some aspect of biotechnology in a readily usable form that almost any teacher can adapt to his/her classroom.

What Do I Need to Know?

For the most part, these lessons assume only a basic familiarity with biotechnology on the part of the teacher. Whenever possible, terms are defined and concepts explained within the lesson or found in the glossary. A teacher who has attended a biotechnology workshop will find it easier to flip through this book and select activities, but a teacher with no workshop experience should be able to make good use of many of the lessons without extensive preparation. The only exception is those lessons involving electrophoresis, where previous experience on the part of the teacher is necessary.

How Is This Book Organized?

This book is divided into five units that attempt to group the lessons by concept. Unlike a workbook, there is no logical progression from one lesson to another. Instead, this is an assortment of activities from which a teacher can pick and choose, like items on a menu. The **introductory section** can serve as a resource for the teacher who wishes to introduce students to biotechnology before undertaking a specific activity. "Biotechnology: An Introduction" and "Biotechnology Careers" can both be used as lecture outlines or photocopied for student reading assignments. The units that follow contain lessons that use hands-on activities. Lessons from **Unit I** can be used to teach the basics of DNA and heredity while **Unit II** lessons focus on the omnipresence of DNA and show students that it can be extracted from any organism and analyzed. The lessons in **Unit III** are designed to illustrate the way that DNA is naturally manipulated by organisms (transformation, conjugation, etc.) and **Unit IV** focuses on how humans can manipulate DNA to achieve particular objectives. **Unit V** addresses the ethical questions that biotechnology has raised and involves students in decision-making exercises. Following the units is an extensive **glossary** of terms and a list of **general resources** to enhance the specific resources listed in each lesson.

What Is "DNA-Man"?

This cartoon character, created by a high school sophomore, is designed to enliven the student pages and help eliminate the impression that molecular biology is too difficult and serious for the average student. He appears in the upper right hand corner of every student page and occasionally within the text.

How Are the Lessons Structured?

Each lesson is made up of teacher pages and student pages. The teacher pages are set in small type on three columns. They contain background information, preparation procedures, lists of materials, answers to student questions and additional resources. The student pages are set in larger type and are designed to be easily photocopied. They contain background information, readings, lab sheets and exercises for the students. Each student page is designated by the presence of "DNA-Man" in the upper right hand corner.

What About Safety?

Several of the lessons involve techniques or chemicals that can be dangerous if mishandled. Please be sure that you and your students follow safe laboratory procedures at all times. Read the procedures carefully and consult state or local safety manuals or textbooks for additional information.

What If I Have a Question?

All the activities have been field tested in a variety of classrooms and reviewed by a number of scientists and educators. In case of a question or comment about a particular lesson, the name, mailing address and phone number of the author is included in each activity. Comments or questions of a general nature are welcomed by NABT or NCBC. The *Sourcebook* is designed as a flexible resource that we hope to revise and expand in the following years. The insight of individual users will be appreciated!

Biotechnology: An Introduction

This can be used as the basis for a lecture or class discussion, or it can serve as an assigned reading. Figures can be used as overheads.

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Introduction

The word "biotechnology" combines two root words: *bio* = life and *technology* = applying science to solve a problem (Figure 1). The word biotechnology refers to a diverse collection of techniques that use living organisms in relatively novel ways to solve problems or make products. Today's biotechnology has three major branches:

- *Genetic engineering* techniques transfer DNA from one organism to another. For example, some plants are being given the genetic codes for pest resistance, and human hormones are being commercially produced by bacteria.
- *Diagnostic techniques* are used to identify specific proteins or DNA. These techniques are being used to detect animal and plant diseases caused by viruses, match DNA at the scene of a crime to a victim or defendant, and monitor the presence of harmful substances in the natural environment or workplace.
- *Cell/tissue techniques* are used to manipulate and grow cells or tissues. Such techniques allow scientists to fuse cell nuclei, cultivate cells for transplants, and transfer embryos from one mother to another.

Biotechnology is currently being used in agriculture, medicine, food processing, bioremediation and energy production. Scientists and industry are researching and developing biotechnological techniques to

- improve the yield, taste, nutrition and disease resistance of plants and animals.
- prevent, test for and treat a variety of conditions and diseases in humans.
- enhance the production and quality of food such as breads, cheeses and fermented beverages.
- conserve natural resources by developing biotechnological alternatives to artificial fertilizers, pesticides and the clean-up of toxic wastes.
- produce fuels and solvents from living organisms.

Despite their potential, biotechnology applications are fraught with difficulties. The complexity of organisms means that research is often slow and costly. As with any new technology, there are certain legal, regulatory and economic constraints that need to be addressed by industry and government. Many of the societal issues surrounding biotechnology are volatile and will require thoughtful dialogue between scientists, businessmen, legislators and the public.

The Three Historical Phases of Biotechnology

1. Until 1797 - Using Whole Organisms

(Figure 2)

Ancient people used organisms for food and to do work. Plants were domesticated and selectively bred for desired characteristics such as taste, shape, nutrition and size. Animals were bred for usefulness as food or for doing work. Without knowing they were using microorganisms, people also made cheeses, beverages and bread using fermentation.

2. 1797 to 1972 - Using Cells and Tissues

(Figure 3)

People entered a second phase of using organisms when they began to understand microorganisms, cells and tissues. In 1797, Edward Jenner used living microorganisms to protect people from disease. Although Jenner did not recognize the existence of microorganisms or the immune system, he used fluids from a person infected with cowpox to immunize people against smallpox. In 1864, Louis Pasteur proved the existence of microorganisms, and showed that all living things are produced by other living things. He used this knowledge to create a rabies vaccine. In 1928, Sir Alexander Fleming discovered the antibiotic properties of certain molds, and by the mid-1940s scientists had developed ways to produce penicillin. Between 1940 and 1972, molecular and cell biologists worked to explain cellular processes at the level of molecules and chemicals.

3. 1972 to future - Using the Chemistry of Cells (Figure 4)

A third phase of biotechnology began in the early 1970s when scientists understood the chemistry of cells well enough to modify the DNA in bacteria. In 1972, Paul Berg and his team isolated a segment of DNA from a virus and combined it with a piece of bacterial DNA. Stanley Cohen and Herbert Boyer built upon Berg's work by placing a small circle of DNA with an extra gene into bacteria, and the new gene functioned normally. Cohen and Boyer called their process recombinant DNA (rDNA). Recombinant DNA techniques are the cornerstone of genetic engineering. The power of genetic engineering is its ability to give researchers *specific* control of organisms' traits and growth. If a gene is identified and isolated, it theoretically can be inserted and expressed in any organism if the proper gene regulatory mechanisms are present.

Biotechnology: An Introduction

Biotechnology Today

When we hear about biotechnology in the news today, the word most often refers to techniques for using the chemistry of cells. Most of these techniques have been developed since the 1970s. Our growing understanding of biotechnology has its "roots" in chemistry, physics, biology and their hybrid disciplines. Look at the "biotechnology tree" illustrated in Figures 5 and 6.

Genetic engineering

Since people began breeding plants and animals, they have wanted to combine the traits of different species. For instance, it would be desirable to move drought-resistant traits from cactus to corn, but cactus will not "cross" with corn. Sexual reproduction normally combines the genes of organisms from the same species, but sexual reproduction cannot usually combine the genes from different species. Genetic engineering is a way to combine and express genes between organisms that do not normally breed.

What happens to an organism when it receives genes from another species? Genes give an organism its traits by directing the manufacture of the organism's proteins. Genetic engineering simply transfers protein factories from one organism to another. The miracle is that genes from any organism can theoretically function in any other organism. Genes are made of DNA, and the DNA molecule appears to be universal.

An example of this universality of DNA is the way that human genes can be used to direct bacteria to make the protein insulin. Insulin is a hormone that controls the amount of sugar in our blood. People with too little insulin have diabetes, a disease that causes blood sugar levels to fluctuate wildly. People with diabetes need extra insulin to maintain their blood sugar level. Before genetic engineering, insulin was extracted from animal tissue, an expensive and somewhat risky source, as animal insulin is slightly different from the human variety and can cause allergic reactions. Bacteria are a much better source because they multiply rapidly and can make large amounts of pure human insulin. After scientists found the insulin gene in human cells it was extracted and placed in bacteria which are now the major producers of insulin.

The techniques used in genetic engineering are:

- *Vector transfer* . This is a way to place a gene into a new cell by special carriers. Such a transfer takes advantage of natural processes that bacteria and viruses use to transfer their DNA, and it involves placing a gene into a sort of "gene taxi" that takes the gene into the host cell.
- *Microinjection* . A microscopic needle or "gun" is used to shoot DNA through a cell membrane into a target cell.
- *Protoplast fusion* . This technique dissolves the cell walls of two different cells so that two cells can combine into one.
- *Electroporation* . Electricity is used to create tiny pores in a cell's outer membrane and move DNA into it.

Some people are concerned that genetic engineers will soon create monsters. Many factors make this unlikely. Genetically engineering a Frankenstein would require the complex manipulation of many genes at once. It is very difficult to identify even the single genes responsible for traits such as production of a hormone. It is still more difficult to know how a gene functions with other genes. Most genes need other genes to turn them on and off. Large and complex groups of genes control the traits of body shape and structure. Scientists know little about how these interacting groups of genes produce traits even in a single tissue. Organs and limbs are often composed of several tissue types, and each tissue is controlled by many genes. Altering body shape or intelligence is well beyond current genetic technologies.

Diagnostic techniques

News about biotechnology might contain words that do *not* refer to genetic engineering. Some biotechnologies use the chemistry of cells to identify proteins or DNA. Two of the most common types of diagnostic techniques are:

- *DNA probes and RFLP's* . These are used to learn if an organism has a specific gene. Scientists use these techniques to detect genes that cause genetic diseases and genetic counselors advise parents who risk having children with genetically transmitted problems. RFLP's are also used by forensic scientists to detect evidence at the scene of a crime or for establishing parenthood of children.
- *Monoclonal antibodies* . These are like the antibodies in our blood: Each antibody is extremely sensitive to a specific organic compound and indicates the presence of that compound to the body's immune system. Monoclonal antibodies are made by special cells that fuse white blood cells and tumor cells to combine the antibody production of white blood cells with the rapid growth of the tumor cells. The term "monoclonal" refers to the fact that the fused cells are "clones," or genetically identical copies made from a single line. The technique is used to make large quantities of antibodies. Such large quantities could not normally be made within one organism.

Cell/tissue techniques

Large numbers of genetically engineered cells are sometimes necessary to make products. The various cell and tissue techniques are used to grow large numbers of cells, cause cells to form specific tissues or transfer living cells from one environment to another:

- *Cell and tissue culture* is a collection of methods for growing cells and tissues from a single cell or a sample of tissue. These techniques are often used to produce large quantities of "clones," or genetically identical copies. Often, these are clones of genetically engineered cells or tissues.
- *Fermentation* is a special kind of large-scale cell culture. Fermentation has traditionally used yeast or bacteria to convert one substance into another (such as sugar into alcohol). Genetic engineers have recently created special cells to make new chemicals, and large quantities are made by growing whole vats

of cells. For example, pharmaceutical companies use fermentation techniques to grow vats of bacteria that produce insulin.

- *Embryo transfer* is a technique for moving identical embryonic cells that are isolated at the 2-,4- or 8-cell stage into one or more other surrogate mothers. The process allows farmers to place one or more potential embryos from a prize cow into many ordinary cows.

Biotechnology in Agriculture

Biotechnology has the potential to affect many aspects of agriculture. Scientists and industry are researching and developing biotechnological techniques to improve the yield, taste, nutrition and disease resistance of plants and animals. Research is also being conducted to improve the post-harvest quality and use of agricultural products. Resource conservation could be enhanced by using biotechnological alternatives to artificial fertilizers and pesticides.

It is natural for agricultural researchers to look for and take advantage of biological answers to the challenges of modern agriculture. Biotechnology research promises to develop organisms and products that cannot be produced as efficiently (or at all) by other technologies.

How can biotechnology help farmers?

Before biotechnology emerged, farmers and scientists relied on selective breeding to produce desirable plants and animals. Although most agricultural products we use today came from selective breeding, it is a laborious and sometimes inefficient process that takes many generations of crossbreeding to obtain a desired trait. Successful crossbreeding also requires that parents are of the same genus or species.

Biotechnology offers techniques to streamline selective breeding. Breeding is still necessary, but with biotechnology it might be possible to add a particular gene to an organism regardless of the origin of that gene. The gene could come from an animal, another plant or even a gene synthesizer. Once a gene is incorporated into an organism, new cell/tissue techniques could reduce the number of generations that were previously necessary to obtain the desirable trait. Biotechnology can help breeders :

- identify genes responsible for specific traits.
- detect desired traits without waiting for organisms to mature.
- transfer traits between organisms that are not genetically similar.
- introduce resistances to pests, diseases and other stresses.
- develop new and improved vaccines for livestock.
- develop pest-resistant crops to decrease the use of herbicides and pesticides.

Are there limits to agricultural biotechnology?

Genetic engineering in agriculture holds promise, especially with respect to traits that are controlled by a *single gene*. However, most traits are under multi-gene control. Multiple gene traits are extremely difficult to engineer and control.

For example, plants need nitrogen to make proteins, but they cannot use nitrogen directly from the air. Roots of peanuts, soybeans, clover and alfalfa contain host bacteria that change ("fix") gaseous nitrogen into a usable form, but plants such as corn must get their nitrogen from fertilizers or from the by-products that other organisms leave in the soil. Nitrogen-fixing corn would reduce fertilizer costs to the farmer, and reduce the amount of nitrates and phosphates that enter aquatic systems from farm runoff. Unfortunately, several problems currently prevent the production of nitrogen-fixing crop plants such as corn. Nitrogen-fixation is controlled by more than 15 different genes in the plant/bacteria system. Many of the involved genes have not yet been isolated. Even if the genes are isolated, scientists must overcome the difficulties of moving the genes into many of the important crop plants such as corn. The genes need to be placed at a particular site in the plant chromosome in order to function, but scientists have not found a way to place desired genes in such an exact location. Even if corn and other important crop plants accepted the nitrogen-fixation genes, it is not known how to turn these genes on and off.

Plant researchers face similar problems in their efforts to genetically engineer improvements in photosynthesis, drought tolerance and other yield-increasing traits. Likewise, animal researchers working to produce better livestock are largely limited to exploring single-gene traits that will not require precise insertion into the animal genome.

Biotechnology in Medicine

How do we cope with disease?

- Prevention
- Testing for disease
- Treatment

What biotechnologies might we use to cope with disease?

- Vaccines made with rDNA methods *prevent* disease.
- DNA probes and monoclonal antibodies *test* for the presence of specific diseases.
- Proteins such as hormones, clotting factors, lymphokines and other drugs are made with rDNA methods for disease *treatment*.

How has biotechnology improved vaccines?

Vaccines prepare our immune systems to defend against a disease before we are exposed to the disease. The immune system recognizes specific substances called antigens that signal the presence of foreign substances such as disease organisms. Vaccines introduce antigens into the body without introducing the living disease organisms. The vaccines cause the immune system to produce substances that will mark future invaders for destruction. For instance, after our immune system is threatened once by the "false alarm" of a hepatitis vaccine, we produce a substance that persists in our blood for many years. The substance will mark hepatitis bacteria so that the immune system will destroy them.

The first vaccines were made from weakened or dead disease-causing organisms. However, vaccines prepared from disease-causing organisms can (very rarely) cause the

disease or other adverse reactions. Safer vaccines are now produced using genetic engineering. The new techniques capitalize on the fact that the immune system needs only to recognize an identifying substance (antigen) on the organism's surface – not the whole organism. Molecular biologists can sometimes isolate the disease organism's antigen-making gene and splice the gene into harmless bacteria or yeast. Pharmaceutical companies grow the harmless antigen-making organism in great quantity and extract the pure antigen. The genetically-engineered antigen is especially safe: It teaches the immune system to recognize the disease organism without introducing that organism into the body.

What are DNA probes, RFLP's and monoclonal antibodies?

DNA probes

These are used to identify and locate genes. Probes take advantage of a characteristic of DNA's basic structure: complementary base pairing. Complementary base pairing results from the fact that the adenine base binds only to thymine and cytosine binds only to guanine. DNA's two joined strands can separate and rejoin and the two strands must go back together just as they came apart. A free-floating single-stranded DNA fragment binds only to its complementary sequence. If it is to bind at all, it must seek out its complementary sequence. Single-stranded DNA fragments can be labeled to exhibit color, antibiotic-resistance or radioactivity. When they bind to their complement, the "label" identifies the target DNA. From previous experience, scientists know that a given probe binds to human DNA near a specific gene. When used with RFLP analysis, DNA probes can pinpoint mutations, establish biological parenthood or identify criminals.

RFLP (riflip) analysis

This process is used with DNA probes to locate genes. The RFLP (restriction fragment length polymorphism) technique detects variations in DNA close to a specific gene on a person's chromosome. The technique uses enzymes to cut DNA near the gene and produce a series of fragments. The fragments are electrically sorted by size to form a distinctive pattern of fragment lengths. The pattern is as consistent and unique for each person as his/her fingerprint. The special fragment patterns occur when one of the two homologous chromosomes differs because of a mutation at a point where a restriction enzyme cuts the DNA, either creating or destroying a cut site. Such a mutation either adds or subtracts fragments from the pattern of restriction fragments. The difference in the homologous chromosomes is called a polymorphism. The identifiable pattern of fragments is called a RFLP. When searching for a gene that indicates a genetic disease, researchers examine fragment patterns of families with the genetic disorder. Eventually, researchers find a special fragment pattern that is usually inherited with the disease. The special fragment pattern is called a "marker." When markers are found, DNA probes are made to identify the DNA in the distinctive fragments.

Monoclonal antibodies

These seek out and bind to specific proteins. They are used in home pregnancy test kits and to diagnose diseases such as AIDS, hepatitis, meningitis and some types of cancer. Monoclonal antibodies can also determine the compatibility of tissues for organ transplants, or serve as the basis for some potential cancer therapies. Antibodies are valuable because they are extremely specific and sensitive to proteins. Each antibody binds to a specific protein, and the antibody will bind even when the protein is present in dilute quantities. Because antibodies are so specific and sensitive, they can be used both inside and outside the body to test for specific proteins. Every organism carries a unique protein signature, so antibodies can reliably identify organisms. Antibodies can even identify pollutants in the soil.

To make monoclonal antibodies, scientists inject a foreign protein or organism into a mouse, stimulating the mouse's white blood cells (lymphocytes) to make antibodies. Normally, the mouse's activated lymphocytes would reproduce slowly and for a short time, producing small quantities of antibodies. Scientists have found a way to fuse the lymphocytes to tumor cells. The fused cells multiply rapidly and can be grown outside the mouse in large containers. The fused cells produce large amounts of the antibody. The antibodies are purified and can be used to test for the protein that was originally injected into the mouse.

How does genetic engineering help produce new drugs?

We have discussed how insulin was made using rDNA techniques. Similar techniques are used to make other drugs including interleukins, interferons, tumor necrosis factor, tissue plasminogen activator (an anti-clotting drug), clotting factors for people afflicted with hemophilia, growth hormones for children afflicted with dwarfism, and drugs to prevent the collapse of babies' lungs. Each of these drugs is a protein produced by a human gene. The DNA code is universal, so scientists can isolate the human genes that produce certain proteins and place the genes in microorganisms such as bacteria and yeasts. The genes function in the microorganisms to produce the human proteins. The microorganisms reproduce in huge numbers, acting as factories for producing the drugs.

Biotechnology in Environmental Science

New organisms may help to manage present and future toxic wastes such as oil spills, farm runoff, fuel storage leaks and industrial chemical leaks. Other organisms may replace harsh chemicals and prevent their use.

Some naturally occurring bacteria consume spilled oil or fuels, but they do so slowly. Waste management companies have developed some non-genetic ways to help the naturally occurring bacteria multiply to numbers that will effectively degrade toxic wastes. Researchers are also developing new genetically engineered bacteria to degrade toxic wastes, but the lack of regulations governing environmental release of genetically engineered organisms has prevented this use of genetic engineering.

Researchers are trying to produce bacteria that would thin heavy crude oil to allow it to flow faster in pipelines. Bacteria can also be used to mine ores or to remove metals

from industrial waste water. Bacteria have replaced the caustic chemicals used in the paper industry, in the poultry industry and in your home.

Plants that produce their own fertilizers and pesticides could reduce the use of agricultural chemicals that pollute ground and surface water. Using more effective forms of bacteria to degrade livestock runoff would decrease the use of chemicals and the amount of livestock runoff reaching our water supplies.

Some people speculate that biotechnology could also help preserve some endangered species. Cloning and tissue culture techniques offer new ways to regenerate plant tissues, and genetic engineering could help to conserve the genes that are disappearing from depleted gene pools.

Some fuels and solvents once manufactured from petroleum products are now manufactured using fermentation and other biological techniques. Brazil now makes much of its automobile fuel by fermenting sugar cane. Biotechnology is likely to improve many of the fermentation processes now in use.

Conclusion

As new techniques are developed and new products become available, the public will need to stay as well informed as possible. It is likely that in the future governments and local communities will need to make choices based on the potential benefits versus the possible risks of biotechnology.

Figure 1

BIOTECHNOLOGY:

using living organisms to solve problems and make products.

In the late 20th century the term refers to techniques used to work with living cells to improve the health, characteristics, applications and productivity of living things.

Figure 2

The Birth of Biotechnology

Using Whole Organisms

Breeding and
<u>Food Processing</u>

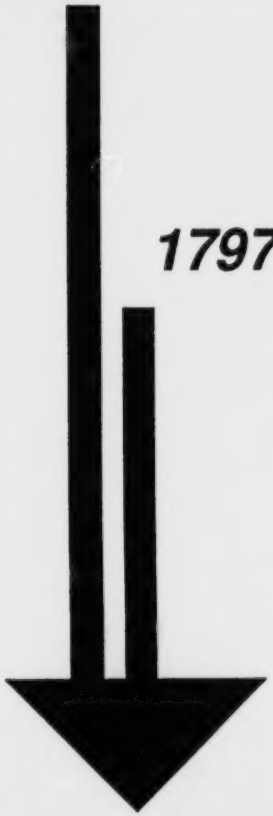
- **Arabian Horses**
- **Corn**
- **Cheese**

The Future

Figure 3

The Birth of Biotechnology

Using Whole Organisms



1797: Using Cells

Vaccines and Antibiotics

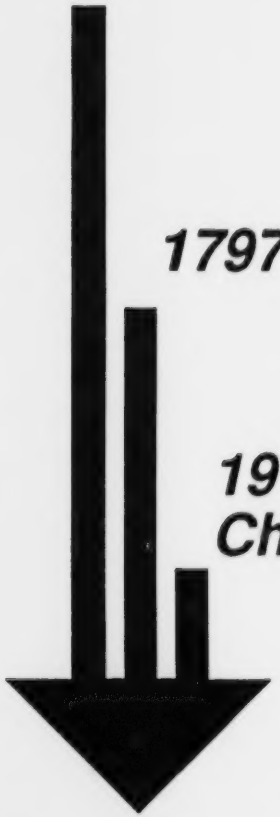
- **smallpox vaccine**
- **penicillin**

The Future

Figure 4

The Birth of Biotechnology

Using Whole Organisms



1797: Using Cells

1972: Using the Chemistry of Cells

The Future

Biotechnology

- **Humulin**
- **genetically engineered tomato**
- **oil-eating bacteria**

Figure 5

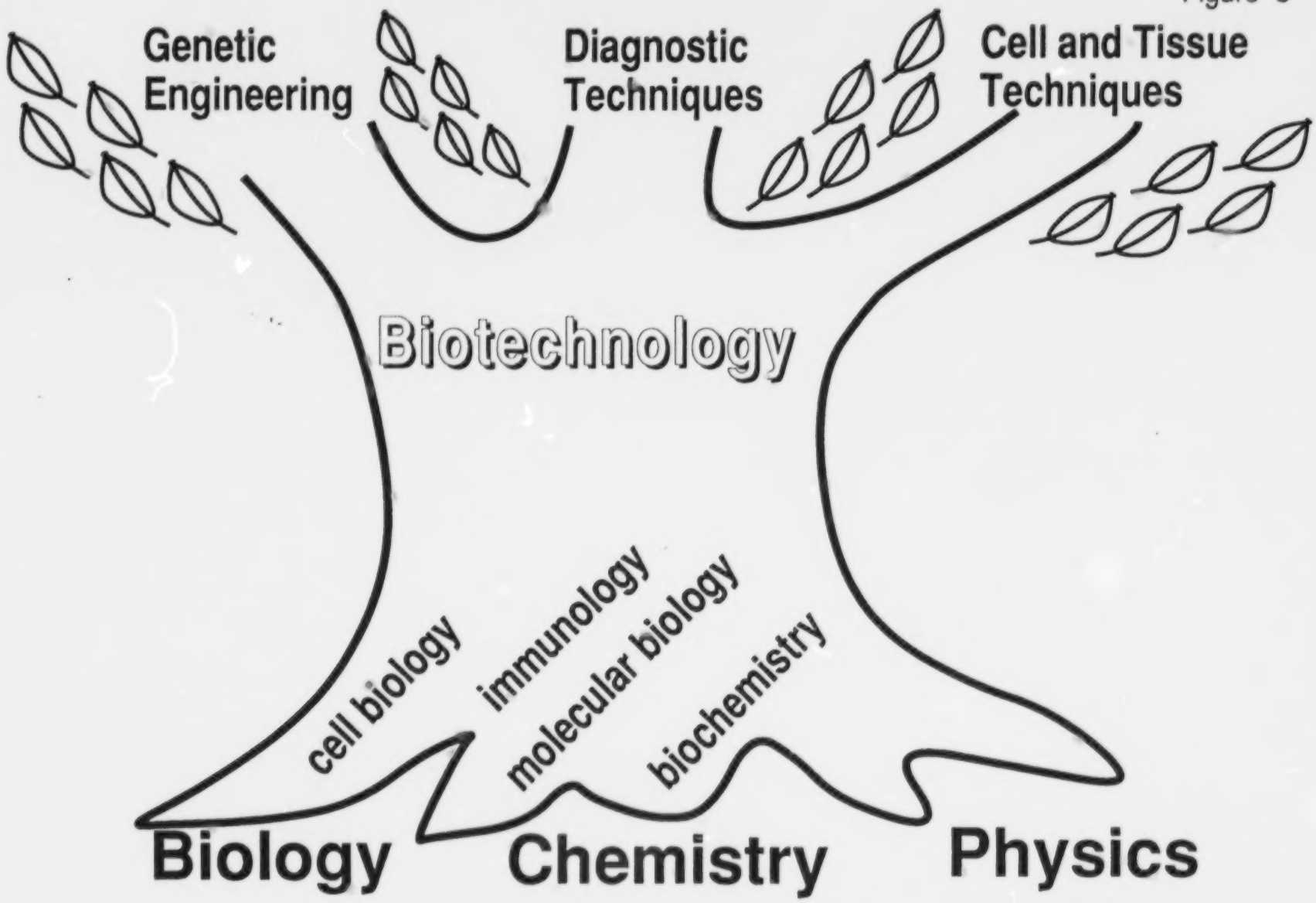

Genetic Engineering
Diagnostic Techniques
Cell and Tissue Techniques
Biotechnology
cell biology
immunology
molecular biology
biochemistry
Biology
Chemistry
Physics

Figure 6

Genetic Engineering

- vector transfer
- microinjection
- protoplast fusion
- electroporation

Diagnostic Techniques

- DNA probes
- RFLPs
- monoclonal antibodies

Cell and Tissue Techniques

- cell & tissue culture
- fermentation
- embryo transfer

Biotechnology

Biotechnology Careers

This can be used as the basis for a lecture or class discussion or as a reading assignment.

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Introduction

Biotechnology is an expanding industry. There are an estimated 60,000 biotechnology-related jobs in America. This number is expected to increase significantly by the year 2000, requiring well-trained people at post-secondary levels from two-year technical degrees to Ph.D.s. Careers in biotechnology offer good salaries, stability and independence.

The Biotechnology Industry

Procedures

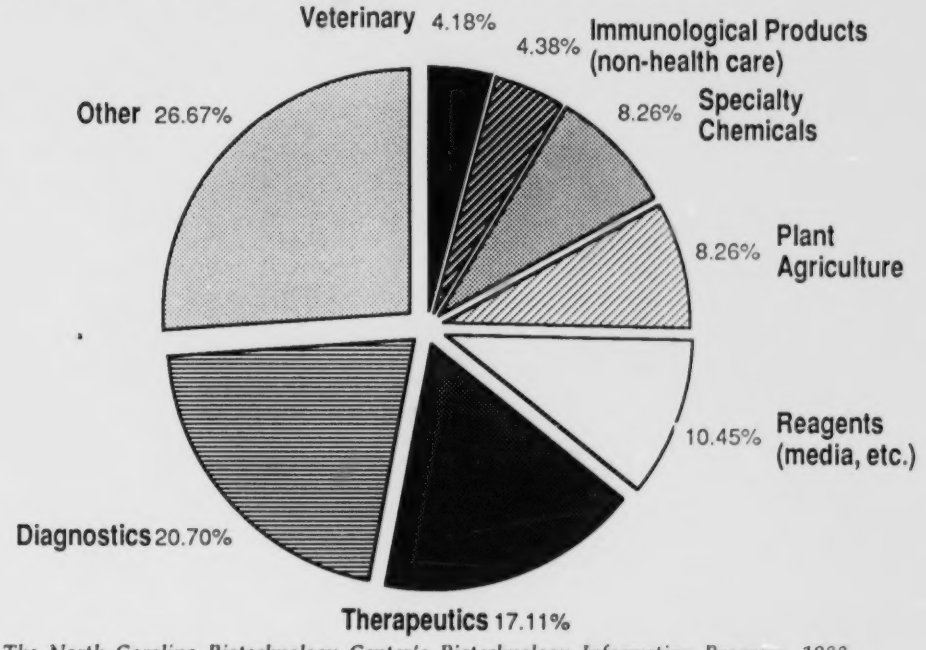
The biotechnology industry is built on three basic technologies:

1. **Genetic engineering**. Any process that involves joining (or recombining) of DNA from one cell or organism to another. Genetic engineering is often referred to as recombinant DNA (rDNA) technology. The goal of genetic engineering is to enable an organism to make a product it would not otherwise produce.
2. **Diagnostic techniques.** Procedures such as monoclonal antibody technology and DNA probes that allow researchers to detect human diseases and conditions, as well as test for food toxins or soil and water contaminants.
3. **Cell and tissue techniques.** Cell fusion, cell/tissue culture, fermentation and embryo transfer are ways to grow and nourish desirable cells or tissues. After biotechnology research has determined a process and a product, engineers design and employ cellular/molecular biology techniques to produce the product in quantity. Many companies license or contract this process work, and some create and operate their own production facilities.

Products

The largest proportion (38%) of the biotech companies produce products for detecting, preventing and treating human diseases (see Figure 1). Scientific supply companies (19%) make specialty chemicals like restriction enzymes or culture media that are used to conduct biotechnology research. Agricultural biotech companies (12%) make new chemicals for plant agriculture or animal vaccines. Non-health care immunological product companies (4%) make monoclonal antibodies to detect a variety of conditions ranging from pregnancies to soil pollutants. The remaining 27% of the biotech companies make products or conduct research in fields such as DNA typing, food technology, mining, waste management and energy.

Percent of Biotech Companies in Various Specialties



Source: The North Carolina Biotechnology Center's Biotechnology Information Program, 1988

Biotechnology Careers

Locations

The biotechnology industry in the United States is concentrated on the Pacific and Atlantic coasts. California-based companies expect to add 40,000 new jobs over the next decade. On the Eastern Seaboard, growth is strong in Massachusetts, New Jersey, New York, Maryland and North Carolina. Employment in biotechnology on the Eastern Seaboard is predicted to rise 250% over the next decade.

Jobs in Biotechnology

Jobs at Universities

University faculty, students and staff working in biotechnology can be found in five major areas: research, production, business, law and medicine.

- *Research*: In universities, biotechnology-related research is conducted principally within the biology and chemistry departments. Students interested in biotechnology might work in specific disciplines such as botany, zoology, molecular biology, biochemistry, analytical chemistry or protein chemistry. In medical institutions, biotechnology-related work might be found in immunology, pharmacology, anatomy, physiology and veterinary medicine.

- *Production*: Biotechnology production programs at universities are primarily in fermentation process engineering. North Carolina State and Duke have biochemical engineering programs employing faculty and researchers.

- *Business*: Biotechnology business programs at universities employ faculty either in general business programs or in a management of technology program.

- *Law*: Law schools employ faculty in specialized programs in patents and regulatory issues.

- *Medicine*: Medical schools involve faculty in clinical testing. Physicians may also become involved in biotechnology research.

Jobs at Biotechnology Companies

Corporations working with biotechnology tend to be well-established and large, and about 60 large U.S. corporations have begun to work in biotechnology. Pharmaceutical companies exemplify the large companies in biotechnology; they average 75 years of age and employ an average of 35,000 people. However, these employment figures could be misleading – only a small proportion of the large companies' employees work with biotechnology.

Nearly 400 smaller U.S. firms are involved with biotechnology, and the majority of these companies' employees work with biotechnology. The average small firm is seven years old with about 90 employees. On the average, 50% of these do research and development, 20% work in marketing, 13% work in management, 10% work in production and 7% are involved with support tasks.

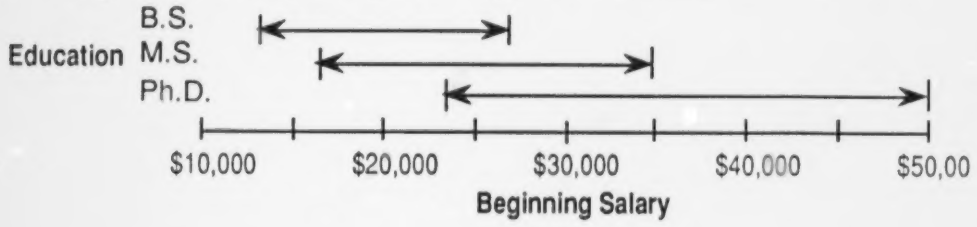
Jobs that Support the Biotechnology Industry

About 1,500 U.S. companies work indirectly with biotechnology. These firms supply biotechnology companies with various services and products. Some law firms specialize in biotechnology patent law; architectural firms specialize in lab design; some firms make equipment such as fermentation vats; and some public relations firms specialize in promoting biotechnology.

Salaries

The following graph reveals beginning salaries for biotechnology researchers in 1987. Wendall Allen and Pamela Meacher of East Carolina University's Molecular Biology/Biotechnology Research and Training Program surveyed 194 public and private companies nationwide to gather the

Beginning Biotech Salary Ranges for Different Education Levels



information summarized below. The survey was published in the September 1988 issue of *Bio/Technology*.

Workers at the B.S. and M.S. levels usually have the title of "research associate" or "associate scientist." *In the biotechnology industry, experience is very important. Some associate-level workers advance to levels of pay and responsibility equivalent to those of some Ph.D. research scientists.* Not shown above is the beginning salary range for technicians with a two-year degree from a technical school. The average starting annual salary for a technician with a two-year degree is approximately $15,000.

The graph below shows the difference between salaries at public institutions (i.e. state universities and national research institutions) and private corporations.

Average starting salaries for public and private companies

The "private" bars on the preceding graph are also appropriate to show the general range of beginning salaries for workers entering careers in *development*. Workers in development might run preclinical trials for pharmaceuticals, field test genetically engineered crops or work on applications for new scientific discoveries. Salaries for careers in biotechnology production range between $25,000 and $35,000 per year. Many of these jobs are for "bioprocess engineers" who design processes and production facilities. Another type of job in this area is for biotechnicians who assist professional scientists, engineers or managers. Biotechnician salaries start between $15,000 and $20,000.

Salaries for careers in biotechnology *management* or *marketing* range between $25,000 and $35,000 annually. People with master's or doctoral degrees tend to start at the top of this range.

Opportunities

The majority of biotech job openings are for workers with a bachelor's degree. Employees with a Ph.D. are also in demand, but far less so than workers with a B.S. Biotech companies require potential employees to have technical skills in five categories: basic lab skills, microbiology, biochemistry/molecular biology, immunology and cell biology. Workers with biochemistry/molecular biology skills are in the highest demand, followed closely by immunology skills. About half of the companies expressed a need for employees with basic lab skills.

Research and development job openings are increasingly available. In 1980, biotechnology firms employed an average of three researchers each; today the average is about 25 researchers. Health care firms working in biotechnology expect to increase their staffs annually by 25%.

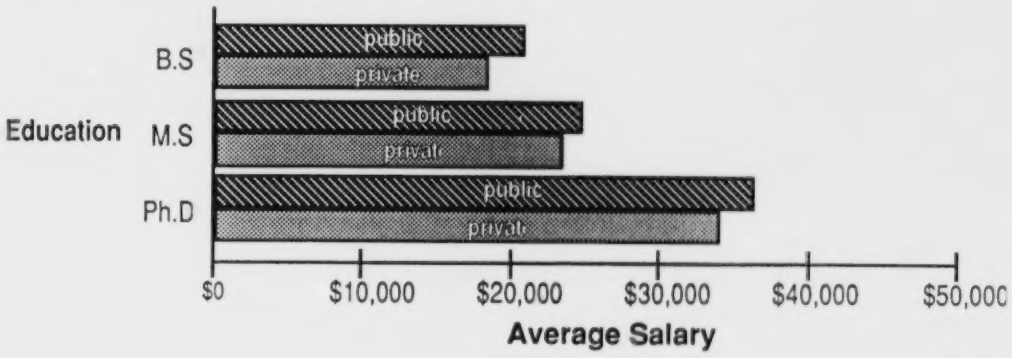
Companies with *production job openings* are actively seeking workers. The U.S. biotechnology industry will need to design processes and production facilities and the industry reports a serious shortage of bioprocess engineers. Analysts say that Japan's lead in this field may seriously damage future U.S. competitiveness in biotechnology. A report from the Center for Occupational Research and Development predicts more than 1,000 new production job openings annually through 1995.

Management and marketing job openings are increasingly available as biotechnology industries move closer to marketing products. There are an estimated 19,500 people working for biotechnology-related firms in management and marketing.

Preparing for a Biotechnology Career

There are four main types of biotechnology careers: *research, development, production and business.* Biotechnology is not a discipline itself, but draws on many disciplines. Preparing for each type of career will require a different combination of disciplines.

Careers in *research* require expertise in a single area of biology or chemistry, such as molecular biology, im-



munology, genetics, biochemistry or protein chemistry.

Careers in *development* also require scientific expertise in biology or chemistry, but focus more on developing and testing products.

Careers in *production* require a combination of biology/chemistry knowledge *and* an understanding of engineering skills and techniques.

Careers in *management or marketing* require knowledge of marketing, finance and/or management as well as knowledge of science and technology.

Careers in biotechnology as researcher, technician, engineer or manager each require different preparation. The table below summarizes the education and skills needed for typical job titles in the four biotechnology career areas.

High school preparation for a research career

High school courses in math, advanced math, biology, chemistry, physics, botany and computers help students

Summary of Education and Skills for Biotech Jobs, by Area

AREA	EDUCATION AND SKILLS	SAMPLE JOB TITLE
Research	formal education in specialized science (M.S. or Ph.D.)	Research Scientist Research Associate Biochemist Enzymologist
	an undergraduate degree in biology or related science, or a 2-year degree at a technical school	Technician
Development	formal education in specialized science (M.S. or Ph.D.)	Molecular Biologist Immunologist Biochemist Enzymologist
	an undergraduate degree in biology or related science, or a 2-year degree at a technical school	Technician
Production	a 2-4 year graduate degree in biology or related science	Botanist Research Assistant Chemical Engineer
	an undergraduate degree in biology or engineering, or a 2-year degree at a technical school	Technician Computer Technician
Management, marketing or other non-science jobs	masters degree from a Management of Technology or MBA program or experience at a biotechnology company	Program Manager Public Relations Marketing Specialist
	undergraduate degree in a general business program	Accountant Salesperson
	graphics, journalism, communication, illustration, media design specialist with 2-4 year degree in journalism, art, media design, or education.	Graphic designer Communications
	an advanced degree in law, accounting, business, or related field, specializing in patents or contracts.	Patent Lawyer Contract Lawyer Financial Analyst

prepare for the undergraduate courses needed to prepare for a research career. Scientific research careers in biotechnology can take many different forms because they may require a combination of expertise in more than one area. Many universities offer four-year and advanced-degree programs in the subjects listed below. Students interested in a scientific biotechnology career should pattern their high school training to qualify for one of the technical school or college/university undergraduate programs listed in the box below.

High school preparation to work as a technician in biotechnology

There will be an increasing demand for technicians by biotechnology companies that begin to commercialize and produce their products. You can work as a technician with a degree from a community college: Last year, 16% of new technicians had AAS degrees from two-year technical schools.

Preparation to work as an engineer in biotechnology

A variety of four-year engineering degrees will prepare students for a career with a biotechnology company. Several universities currently offer work in bioprocess (or biochemical) engineering.

Preparation for a management career in biotechnology

Management careers in biotechnology require skills in both business and science. High school business courses, along with biology and chemistry classes, are recommended. Two- or four-year college majors include business, business management, accounting, economics, marketing and design. Advanced degrees include business, law and finance.

Conclusion

A glance through the classified employment ads in any major newspaper will illustrate the wide range of jobs available in biotechnology. It is important to stay informed about the development of new technologies and products so as to be prepared when exciting career opportunities present themselves.

Undergraduate Programs

Agricultural Sciences	Chemistry	Microbiology
Biochemical Engineering	Enzymology	Veterinary Medicine
Biology	Genetics	Virology
Botany	Immunology	Zoology
Computer Science	Molecular Biology	

The Size of the Genome

Ability level ..... Grade 7 life science – Grade 12 AP biology
Class periods required ..... 1/2 – 1

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Introduction

Teaching about genes as "things" requires special measures. Students have difficulty grasping the incredibly small scale of biology at the molecular level, so they need ways to imagine what they cannot see. How big are bacteria? How long are their strands of DNA? How big is a gene? What is a plasmid? How big are these things in relation to each other? The answers require more than words and numbers.

Scientists use bacteria as a model system for studying the molecular genetics of more complex multicellular organisms. Bacterial DNA is easier to study because it is not surrounded by a nuclear membrane; instead, the bacterial chromosome is a circle of DNA that is anchored in the cell membrane. In addition, the amount of DNA found in bacteria is much less than the amount found in multicellular organisms. The biotechnology techniques related to genetic engineering, monoclonal antibodies, DNA probes and cell/tissue culture all require an understanding of the size relationships among DNA, genes, plasmids and chromosomes.

This activity demonstrates various size relationships among genes, bacterial chromosomes, plasmids and bacterial cells. Students will also learn about some of the physical properties of DNA and the sizes of bacterial genomes compared to the human genome.

Objectives

At the end of this activity, students should be able to:

1. Describe the relative sizes of bacterial cells, chromosomes, genes and plasmids.
2. Explain that strands of DNA are extremely thin and must be very tightly coiled; an immense length of DNA fits into a relatively tiny cell volume.
3. Explain the differences between bacterial and human genomes.
4. Recognize and understand these words: **DNA, genome, plasmid, bacterium, chromosome, gene, supercoiled DNA, prokaryote.**
5. **(Optional)** Use scientific notation to confirm the dimensions for bacterial DNA.

Materials

For entire class

- felt tip pen
- scissors
- glue
- meter stick or metric ruler

For each lab team of four students

a letter-size envelope containing:

- a 2 cm (20 mm) gelatin capsule ("No. 0"; these capsules can be purchased at pharmacies or health food stores)
- 10 meters of white thread; 10 meters of colored thread
- 220 mm strands of colored yarn or twine; red and blue *or* choose your school colors!
- 2 blank 3"x5" index cards

Teacher Preparation

Make a demonstration kit for yourself and one for each lab team

1. Place one gelatin capsule into each envelope.
2. Use a meter stick or ruler and scissors to cut the two pieces of colored yarn or twine and the sewing thread to the following lengths:
   - 1 length of white thread and 1 length of colored thread – 10 meters each
   - 1 piece of blue yarn or twine – 4 millimeters long (This will be just a piece of blue "fuzz" if you use yarn.)
   - 1 piece of red yarn or twine – 10 millimeters long
3. Put the two 10-meter lengths of thread together and coil around your hand. Each length of thread represents one side of the DNA double helix.
4. Glue the 4-millimeter piece of blue yarn or twine to a 3"x5" card and label it "Length of DNA in a single bacterial gene."
5. Glue the 10 mm piece of red yarn to a 3"x5" card and label it "Bacterial plasmid." If you use thread or string, glue it into a circle on the card to simulate the circular nature of plasmids.
6. Put all materials from Steps 2-5 into each envelope.

Teaching Tips

1. If time allows, have your students prepare the "gene" and "plasmid" cards, and measure out the lengths of thread (Teacher Prep, steps 2-5). This enables them to practice metric measurement.
2. The student questions involving scientific notation should be assigned relative to the math abilities of your students.

Procedure

1. Students do background reading. (You may want to go over it with them in class.)
2. Students split into groups of four and follow the procedure on the activity sheet. (In step # 7, the best way to fit the thread in the capsule is to fold the lengths of thread in half several times until they have a "wad" of thread approximately 3 inches long. Insert one end of the folded thread into the longer section of capsule. Push the "wad" into the capsule with a twisting motion.)
3. Students do the question sheet. (This can be in class or as homework.)

The Size of the Genome

Answers to Student Questions

1. *Use a microscope or make a model you can see.*

2. *Bacteria contain DNA just like other living cells. The main portion of bacterial DNA is contained in its singular* ***chromosome****. The DNA segments of the chromosome that code for a specific protein are called* ***genes****. Bacteria also contain circular, non-chromosomal pieces of DNA called* ***plasmids****.*

3. *Each length is one side of the DNA helix.*

4 *If each gene is 4 mm long there would be 250 genes in a meter, or 2,500 in 10 meters. Actually, some genomic DNA is "nonsense." Most bacterial genomes contain only 2,000 genes that code for proteins..*

5. *This activity reinforces the super-coiling nature of DNA and the extreme smallness in diameter relative to its length that DNA must possess.*

   Have your students fold the lengths of thread in half several times until they have a "wad" of thread approximately 3 inches long. Insert one end of the folded thread into the longer section of capsule. Push the "wad" into the capsule with a twisting motion. The thread will fit into the capsule, but obviously takes up more (approx. 90%) than 10% of the volume.

6. *10.2 m to represent the E. coli genome.*

   10.2 x 1,000 = 10,200 m to represent the human genome

   10,200 m divided by 1,609 m/mile = 6.3 miles to represent the human genome in miles.

   Note: **Remember that we have expanded linear lengths** by 10,000X. An extension of this activity would be for the students to calculate real linear lengths of DNA. Merely divide values above by 10,000.

7. $3.0 \times 10^6 \times 3.4 \times 10^{-10} m = 10.2 \times 10^{-4} = 1.02 \times 10^{-3} m$.
8. $1.2 \times 10^3 \times 3.4 \times 10^{-10} m = 4.08 \times 10^{-7} m$.
9. $3.0 \times 10^3 \times 3.4 \times 10^{-10} m = 10.2 \times 10^{-7} = 1.02 \times 10^{-6} m$.
10. $1.02 \times 10^{-3} m \times 1.0 \times 10^4 = 1.02 \times 10^1 m = 10.2 m$.
11. $4.08 \times 10^{-7} m \times 1.0 \times 10^4 = 4.08 \times 10^{-3} m = 4.08$ mm.
12. $1.02 \times 10^{-6} m \times 1.0 \times 10^4 = 1.02 \times 10^{-2} m = 10.2$ mm.
13. *3 x 10^9 ties x 2 ft per tie = 6 x 10^9 ft. 6 x 10^9 ft = 1,136,363.6 miles (6x10^9 ft divided by 5,280 ft/mile). = 1,829,545.5 kilometers (1,136,363.6 miles x 1.61 km/mile).*
14. *The length (1,136,363.6 miles) of the genome divided by the equatorial circumference (24,000 miles) will equal 47.3 circuits around the earth.*
15. *Will vary depending on the size of your students' textbook.*

The Size of the Genome

STUDENT PAGE 1

Background Reading

The Genome

All organisms, no matter how simple they may seem to us, require a substantial amount of DNA to carry the instructions necessary to live and reproduce. This DNA is referred to as the "genome." Because DNA is microscopic, it's hard for humans to visualize the actual size of the genome. This is especially difficult when the organism itself is tiny, such as the bacterium *E. coli*. A single *E. coli* bacterium is about a millionth of a meter (1-2 microns) long. Scientists who study genes have used *E. coli* in many of their experiments, so it is a good organism for us to use as a model today.

The Size of *E. coli*

In this activity you will make a model of an *E. coli* cell that will be 10,000X bigger than actual size. (This is comparable to inflating an ant to the size of a tractor trailer.)

- An *E. coli* bacterium expanded 10,000X would be approximately 20 millimeters long, and look like:

a small pill →

The Size of *E. coli's* DNA

In bacteria, the DNA is a large circle. It accounts for 2-3% of the cell's weight and occupies about 10% of its volume. The remarkable features of DNA are its length and slenderness. If we enlarged the DNA of an *E. coli* 10,000X, its total length would be 10 meters around. A loop that length would go around a large inflatable swimming pool. If you cut the loop and straightened it into a line, the strand would be two car lengths long. Imagine how slender the 10-meter strand must be to fit in only 10% of the space in the bacterium model!

A DNA strand of two car lengths

fits into

10% of the space in the bacterium model

20 mm

The Size of *E. coli's* Plasmids

In many recombinant DNA experiments, biologists use a tiny circlet of bacterial DNA that is much smaller than the length of chromosomal DNA. This circlet is called a plasmid. Plasmids occur naturally and are small enough to pass through living bacterial cell membranes. As one might expect, plasmids contain only a few genes, none of which is essential to the bacteria. (In contrast, *E. coli's* loop of DNA contains about 2,000 genes). Molecular biologists add desired genes to plasmids, then insert the new plasmid with the added gene into a living bacterium. Plasmids are considered to be "gene taxis," and are often called vectors.

An *E. coli* plasmid enlarged 10,000X would be about 10 millimeters in circumference (a circle 3.2 mm in diameter), about the size of the plasmid in the center below. If you cut the plasmid and straightened it into a line, the strand would be about one-half the length of a bacterium. The plasmid would also be extremely thin. Floating in the bacterial medium, the plasmid can collapse and coil like a very fine rubber band.

Bacterium	Plasmid	Linear Plasmid
20 mm	3.2 mm	10 mm

The Size of *E. coli's* Genes

Genes are lengths of DNA that code for a specific protein. A gene can be a continuous length of DNA, or it can be many different pieces of DNA interspersed along a chromosome. Researchers in the early 1970s began the age of recombinant DNA (rDNA) by identifying and isolating genes that they wanted to express in *E. coli*. Once the genes were identified and isolated, they were packaged into plasmids. The altered plasmids were inserted into living bacterial cells, and the proteins coded for by the "new" genes were expressed by the bacteria. A bacterial gene enlarged 10,000X would be approximately 4 mm in length.

Bacterium	DNA in one gene
20 mm	4 mm

The Size of the Genome

STUDENT PAGE 2

Activity Sheet

Materials

(Obtain from your teacher)
a letter-size envelope containing:

- a 2 cm (20 mm) gelatin capsule
- 10 meters of white thread; 10 meters of colored thread
- a 3"x5" index card labeled "Bacterial gene"
- a 3"x5" index card labeled "Bacterial plasmid"

Get together with three of your classmates to make a group of four for the next three steps:

Procedure

1. Remove the gelatin capsule from the envelope. It represents a single *E. coli* bacterium that has been enlarged 10,000X.
2. Remove the two 3" x 5" cards from the envelope. The lengths of yarn on the cards represent the lengths (and not the *diameter*) of an *E. coli* gene and plasmid magnified 10,000X.
3. Remove and stretch out the two lengths of thread in the envelope. These represent the bacterial chromosome.
4. Two people should make a circle with the two threads. This represents the bacterial chromosome which is simply a circle of DNA that is attached to the cell membrane.
5. A third person now holds up the 3"x5" card labeled "Length of DNA in a single bacterial gene" next to the DNA "loop." The 4 mm long piece of yarn glued on the card represents the length of a single gene. Remember, a gene is composed of all the segments of DNA that instruct the cell to make a single protein, whether these segments are continuous or not.
6. With the white thread loop and the card labeled "Bacterial gene" still in view, ask the fourth person to hold up his/her 3"x5" card labeled "Bacterial plasmid." The 10 mm piece of yarn glued on this card represents a typical bacterial plasmid. Plasmids carry several genes necessary for their own replication and stability, but you can see that the plasmid is tiny in comparison with the bacterial chromosome. Plasmids can also carry genes that add important characteristics (such as antibiotic resistance) to a bacterium.
7. Now that you have compared the sizes of the chromosomes, plasmids and genes, try to construct the bacterium. This means that the long lengths of thread representing the chromosome have to go inside the capsule. It's not easy!

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The Size of the Genome

STUDENT PAGE 3

Questions

About the activity:

1. Bacteria are difficult to study because of their extremely small size. One thousand average-sized bacteria linked together like a strand of beads would only be as big as the period at the end of this sentence. How can one study bacteria if they are so small? Propose two solutions to this question.

2. Discuss how the following bacterial structures relate to each other: bacteria, DNA, chromosome, gene, plasmid.

3. Why does it take *two* lengths of thread to represent the chromosome?

4. How many bacterial genes would fit on your DNA circle (formed in Step #4)?

5. Is the thread that you tried to stuff in the capsule too thick to represent the DNA's actual thickness? Hint: what % of bacterial cell volume is your thread using, and what is the actual volume that DNA occupies in bacteria?

6. In this activity, how many meters did it take to represent the *E. coli* genome? If the human genome is 1,000X greater than the *E. coli* genome, how many meters would it take to represent the human genome? How many miles to represent the human genome?

Mathematical calculations of bacterial and human genomes:

- The distance between DNA base pairs is 3.4×10^{-10}m.
- The DNA chromosome in *E. coli* has about 3 million base pairs; the average gene has 1,200 base pairs; and the average plasmid has about 3,000 base pairs.

Using the information above, calculate the following:

7. How long (in meters) is the DNA strand in *E. coli*?

8. How long (in meters) is the average *E. coli* gene?

9. What is the circumference (in meters) of an average-sized *E. coli* plasmid?

10. If *E. coli* is magnified 10,000X normal size, how long (in millimeters) is its DNA strand?

11. If *E. coli* is magnified 10,000X normal size, how long (in millimeters) is a gene?

12. If *E. coli* is magnified 10,000X normal size, how long (in millimeters) is a plasmid?

The human genome can be related to a length of railroad track. Imagine that you have uncoiled and untwisted the DNA molecule in a single human cell.The ties will represent base pairs and the rails will represent the deoxyribose sugar and phosphate sides of the DNA molecule. The railroad ties are approximately two feet apart.

13. Remember that there are 3×10^9 base pairs in the human genome. How many miles (or kilometers) of track will it take to represent a human genome?

The Size of the Genome

STUDENT PAGE 4

14. How many miles (or kilometers) of track would wrap around the earth at the equatorial circumference = 24,000 miles)?

15. Your textbook can be used to relate the number of characters (letters and spaces) on a page to the number of base pairs ($3x10^9$) in the human genome. How many books like your textbook would be needed to represent the human genome?

Procedure

- Choose a page in your textbook that is mostly printed.
- Count the number of characters on five randomly selected lines. Calculate the average number of characters per line **(C)**.
- Count the number of lines on the page **(L)**. Multiply the average number of characters on a line times the number of lines on the page. **(C x L)**
- Multiply the number of characters for a page by two **(2)**. This will give you the approximate number of characters for a full page (front and back). **(C x L x 2)**
- Divide the total genome of a human cell by the number of characters on a full page in the textbook. This will give you the number of pages that will be needed to represent the human genome. **(3×10^9)** divided by **(C x L x 2) = (P)**.
- Divide the number of pages needed for a human genome by the number of pages in your textbook **(T)**. This will tell you number of books **(N)** that would be needed to record the information in the genome of one human cell.
  (P) divided by **(T) = (N)!**

How Genes Make Proteins

Ability level ..... Grade 7 life science – Grade 12 AP biology
Class periods required 1–3

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Introduction

This lesson examines how the sequence of nucleotide bases in DNA are translated into a sequence of amino acids in proteins. Students will act out the steps of protein synthesis to learn about this process. The lesson can be used as a follow-up to a lesson on DNA structure.

Objectives

At the end of this activity, students should be able to:

1. Name five types of proteins and describe their functional roles.
2. Define and describe the two major processes of protein synthesis, including:
   - where these processes occur and the major cellular structures involved.
   - the roles of DNA, mRNA, tRNA and amino acids.
3. Use any two sequences of DNA and mRNA, or mRNA and tRNA to demonstrate the concept of complementary base-pairing.
4. Recognize and understand these words:
   protein synthesis, template, complementary base pairing, amino acids, nucleotides, genetic code, transcription, translation, DNA, mRNA, tRNA, codon, anticodon.

Materials

For entire class

- photocopy masters from the *Sourcebook* (4 DNA, 4 mRNA, 4 tRNA and 2 amino acid)
- colored paper (card stock if possible), four colors, two pages of each color
- scissors
- letter-sized envelope

Adapted from Luken, J. (1987). Does biotechnology have a place in introductory biology. *The American Biology Teacher*, 49(6).

Teacher Preparation

1. Use the photocopy masters and colored paper to make the sheets from which the cards can be cut. The master sheets need to be photocopied as two-sided pages. The first two master sheets make one page, sheets 3-4 make the second page, etc. You should have two pages of DNA, mRNA and tRNA, and one page of amino acids when you finish.
2. Cut out the code cards from the sheets (or have your students cut them out in class). Arrange the cutouts into four stacks: DNA, mRNA, tRNA and amino acids. Keep the "start" sequence (TAC) on top of the DNA stack, and the stop sequence (ATC) on the bottom.
3. Store the cards in an envelope.

Teaching Tips

1. Laminate the four stacks of cards for use with your other classes.
2. Multiple copies of the cards can be used for quizzes, remedial flash cards or make-up challenges.

Procedure

1. Students do Background Reading. You may wish to discuss this as a class.
2. Students follow the procedure on the Activity Sheet. They set up the classroom, then act out Transcription and Translation.
3. Students complete Question Sheet (can be a homework assignment or done as a class).

Answers to Student Questions

1. *Collagen, keratin – structural*
   Hemoglobin, serum albumin – transport
   Hormones (auxins, gibberellins, insulin) – chemical messengers
   Enzymes (RNA polymerase, amylase) – catalyze biochemical reactions
   Actin, myosin – contractile, found in muscles
   Antibodies – fight foreign substances in the body
2. ▼ mutations, selective breeding and genetic engineering:

Compare: All are mechanisms of genetically modifying organisms.

Contrast: Mutations occur naturally (or can be induced), can occur as a change in a single base pair, gene or whole chromosome; selective breeding is man-made and occurs at the organism level; does not change genetic make-up of organisms involved in breeding event, but alters the genetic makeup of the population or gene pool; genetic engineering is man-made and occurs at the base pair or gene level.

▼ DNA replication and protein synthesis:

Compare: Both require an unzipping of the DNA molecule.

Contrast: The function of DNA replication is to make more DNA, occurs in the nucleus; the function of protein synthesis is to make proteins, occurs partly in the nucleus and partly in the cytoplasm.

▼ transcription and translation:

Compare: Both are major processes of protein synthesis.

Contrast: Transcription occurs in the nucleus, involves DNA and mRNA; translation occurs in the cytoplasm, involves mRNA, tRNA and amino acids.

3a. *Uracil in RNA substitutes for thymine in DNA*

b. *The order of nucleotides in DNA dictates the order of nucleotides in mRNA. The linear sequence of codons in the mRNA dictates the order of tRNAs. Since specific amino acids are attached to specific tRNAs, DNA also dictates the order of amino acids. Amino acids are the building blocks of proteins.*

4. *Translation. Because transcription involves DNA and mRNA, and can occur without the presence of a particular amino acid. Translation depends on the presence of amino acids to build proteins.*

How Genes Make Proteins

5a. *They are exact opposites of each other.*
b. *Yes. Because the opposite message would be transcribed and translated, and the desired protein not produced.*

6a.

Use the following tRNA/amino acid relationships:	GGC pro	UUA asn	CAG val	CUC glu	GAU leu	AGG ser	CCG gly

DNA:	TAC	AGG	GGC	CTC	TTA	CAG	CTC	GAT	AGG	CCG	GAT	ATC
mRNA	*AUG*	*UCC*	*CCG*	*GAG*	*AAU*	*GUC*	*GAG*	*CUA*	*UCC*	*GGC*	*CUA*	*UAG*
tRNA	*UAC*	*AGG*	*GGC*	*CUC*	*UUA*	*CAG*	*CUC*	*GAU*	*AGG*	*CCG*	*GAU*	*AUC*
Amino Acid	*met* **start**	*ser*	*pro*	*glu*	*asn*	*val*	*glu*	*leu*	*ser*	*gly*	*leu*	**stop**

6b. *They are the same except for "T" and "U."*

7.

Use the following tRNA/amino acid relationships:	GGC pro	UUA asn	CAG val	CUC glu	GAU leu	AGG ser	CCG gly

DNA	TAC	GAT	CAG	GGC	CCG	TTA	AGG	CTC	CTC	GGC	CAG	ATC
mRNA	*AUG*	*CUA*	*GUC*	*CCG*	*GGC*	*AAU*	*UCC*	*GAG*	*GAG*	*CCG*	*GUC*	*UAG*
tRNA	*UAC*	*GAU*	*CAG*	*GGC*	*CCG*	*UUA*	*AGG*	*CUC*	*CUC*	*GGC*	*CAG*	*AUC*
Amino Acid	met **start**	leu	val	pro	gly	asn	ser	glu	glu	pro	val	**stop**

Resources

Kits

Demonstration kit: *DNA Made Easy.* Catalog #17-1040. Carolina Biological Supply Company (2700 York Rd., Burlington, NC 27215).

Audio/Visuals

6-part videotape: *Protein Synthesis.* Catalog #84-2489. TV Ontario (TV Ontario, 143 W. Franklin St., Suite 206, Chapel Hill, NC 27514).

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How Genes Make Proteins

STUDENT PAGE 1

Background Reading

Naturally occurring **mutations** have created new genetic combinations since the origin of life. For centuries, humans have developed and used **selective crossbreeding** to improve organisms used for food, clothing, transportation, etc. Since the early 1970s, **genetic engineers** have developed molecular techniques to alter the genetic make-up of organisms. All three of these mechanisms involve changing the genetic make-up of organisms. How do these changes in an organism's genetic make-up (genotype) affect the trait that is expressed (phenotype)?

Proteins provide the structural and functional basis of life. They play a part in every conceivable life function:

- A structural protein called collagen helps make up cartilage and tendons. Another protein (keratin) is found in our hair and fingernails.
- Hemoglobin is a transport protein that carries oxygen through the body.
- Plant hormones such as auxins and gibberellins are proteins that enhance or regulate biochemical messages. Insulin stimulates our blood to remove sugar.
- Proteins that catalyze chemical reactions in organisms are called enzymes. Amylase helps us to digest starches, and RNA polymerase assists with the transcription process that you will learn about in this activity.
- Proteins also perform many other functions, serving as antibodies, nutrient and waste transporters, nutrient storers, receptors and contractile proteins for muscles.

Proteins have different functions, activities, shapes and chemical nature. Proteins are long, chainlike molecules that assume twisted 3-dimensional shapes. If we could see a typical protein, it might look like this:

Each link of a protein chain is a simple organic unit called an **amino acid.** There are 20 amino acids that are used to form protein chains. The proteins we eat are broken down and then rearranged into the proteins we need.

DNA, a type of nucleic acid, is a long, double-stranded molecule made up of units called **nucleotides.** One nucleotide consists of a deoxyribose sugar, a phosphate group and one nitrogenous base. The sequence of nucleotides contains information necessary for making a chain of amino acids – a protein chain. That sequence of nucleotides is called a gene. Sometimes several DNA sequences work together to make a protein; a gene is not always one continuous stretch of DNA.

Protein synthesis involves two basic processes, transcription and translation, that make use of another nucleic acid, RNA. RNA, like DNA, is made up of a chain of nucleotides. In transcription, enzymes catalyze the transfer of DNA's information to messenger RNA (mRNA) molecules. The mRNA molecules then move out of the nucleus to the ribosomes, where protein synthesis occurs.

Translation is the process of decoding the transcribed DNA message contained in mRNA. A second type of RNA, a cloverleaf shaped molecule called transfer RNA (tRNA) is involved. In the cytoplasm of the cell, specific tRNAs attach to their particular amino acid. At the base of each tRNA molecule is a sequence of three nucleotides (anticodon) that will recognize a complementary set of three nucleotides on the mRNA molecule (codon). The tRNAs, bonded to their amino acids, move to the ribosome where the mRNA is attached. The tRNA's and mRNA's bond together, as do the amino acids. The mRNA's and tRNA's release from each other, and the sequence of amino acids that is left is what defines the protein.

In this activity you will act out the steps of transcription and translation in protein synthesis.

How Genes Make Proteins

STUDENT PAGE 2

Activity Sheet

In this activity you will act out the steps of transcription and translation in protein synthesis.

Procedure

A. Setting the scene

▼ The classroom's floor, walls and ceiling are analogous to a cell membrane. The windows and doors represent the membrane's selectively permeable pores because they regulate the size of the objects that can enter or leave the "cell." (It would be hard for an elephant to come into the room!)

▼ Designate one area of the class as the "nucleus," where **transcription** occurs. Enclose it with chairs and desks to represent the nuclear membrane. Spaces between chairs and desks simulate nuclear pores that regulate the movement of mRNA in and out of the nucleus.

▼ All other non-nucleus areas in the room are "cytoplasm."

▼ In the cytoplasmic region designate an area of the class as the "ribosome," where **translation** occurs.

1. The teacher will distribute the DNA sequences and their complementary mRNA codes to two groups of students. The cards are arranged in groups of three letters because the nitrogenous bases of the *genetic code* function as triplet-base units. The large letters on the cards refer to first letter of the nucleotide bases (A=adenine, C=cytosine, G=guanine,T=thymine and U=uracil). The nucleotide base "thymine" found in DNA is replaced by the base "uracil" in all RNA molecules. Do not distribute the tRNA and amino acids cards until step #8.
2. Review DNA structure and the concept of complementary base-pairing. Recall that the informational part of DNA is within the sequence of nitrogenous bases. Protein synthesis, just like DNA replication, does not begin until a stretch of DNA gets the signal to "unzip" and expose the nitrogenous bases.

B. TRANSCRIPTION: The DNA message is *transcribed* into mRNA by the enzyme RNA polymerase.

1. Assume that a strand of DNA has unzipped, exposing DNA's bases. In reality, one of the two strands is "active," while the other acts as a "dummy." You will be working with the active strand in this activity.

2. Students with DNA cards should line up in the classroom area designated "nucleus." The student with the DNA card labeled "TAC" (the start sequence) should be on the left as the class sees him/her, and the "ATC" (the stop sequence) card should be on the right. All other DNA cards can be arranged in any order.
3. RNA polymerase catalyzes the pairing of DNA's exposed bases with complementary RNA bases. (Remember, only one of the two DNA strands is active.) Students with the RNA cards should match their 3-letter sequence with the 3-letter sequence of the DNA cards. The 3-base mRNA sequence is called a codon.
   ▼RNA cytosine always pairs with DNA guanine.
   ▼RNA uracil (Remember: "U" substitutes for "T") always pairs with DNA adenine.
   ▼RNA adenine always pairs with DNA thymine.
   ▼RNA guanine always pairs with DNA cytosine.
4. After everyone matches up their cards, use the following table to check the DNA/RNA pairs. Notice that of the nine pairs, the stop and start pairs are the only ones that must be in a certain position.

DNA	TAC	GGC	TTA	CAG	CTC	GAT	AGG	CCG	ATC	
mRNA	AUG start	CCG	AAU	GUC	GAG	CUA	UCC	GGC	UAG stop	*Transcription*

How Genes Make Proteins

STUDENT PAGE 3

5. Students with the DNA cards can sit down, leaving a chain of RNA sequences. You have simulated the process of **transcription** as it happens in protein synthesis. Notice you have made a very short (shorter than in real life) **complementary** section of RNA that almost reflects the exact opposite of the DNA code.

C. TRANSLATION: The mRNA message is *translated* into a chain of amino acids called a protein via enzymes and tRNA.

Notice that the tRNA cards are also arranged in groups of three letters. The three-base sequence of tRNA is called an *anticodon*. In addition, each tRNA card has a 3-letter abbreviation (in the "arrow" part of the card) for one of the 20 amino acids. This activity includes only seven of the 20 protein-building blocks called amino acids.

1. If there are enough students, distribute the tRNA and amino acid cards to two new groups of students. Students with either type of card should be randomly scattered in the "cytoplasm."
2. Students with the tRNA cards should find their specific amino acids. For example, the tRNA anticodon card "GGC" with the letters PRO should find the amino acid PROLINE. Simultaneously, students with mRNA cards should walk out the "nucleus" through the rows of desks/chairs that represent the nuclear pores and stop in the area designated "ribosome."
3. After the tRNA students find the students with amino acid cards, *both students* should proceed over to the mRNA card that matches the tRNA card. Use the rules of complementary base-pairing:
   - ▼ RNA cytosine always pairs with RNA guanine.
   - ▼ RNA uracil always pairs with RNA adenine.
   - ▼ RNA adenine always pairs with RNA uracil.
   - ▼ RNA guanine always pairs with RNA cytosine.
4. Messenger RNA and tRNA anticodon sequences are given below based on the original DNA sequence given in step #7. Except for the start and stop cards, student cards will not necessarily be in this order across the row, but they should match up vertically (e.g., mRNA's "CCG" will pair with tRNA's "GGC"). Use the following table to check the mRNA/tRNA pairs:

mRNA	AUG	CCG	AAU	GUC	GAG	CUA	UCC	GGC	UAG	
tRNA and amino acid	UAC met	GGC pro	UUA asn	CAG val	CUC glu	GAU leu	AGG ser	CCG gly	AUC stop	*Translation*

5. As each tRNA anticodon finds its corresponding codon on the mRNA strand, the tRNAs detach from their amino acids. The amino acids remain at the ribosome and form a peptide bond with the amino acid brought by the previous tRNA. Two or more amino acids linked in this way are called polypeptides. *Translation* is complete when a sequence of mRNA information translates into a polypeptide. A protein is one or more polypeptide chains linked together.

How Genes Make Proteins

STUDENT PAGE 4

Questions

1. Name five types of proteins and describe their functional roles.
   a.
   b.
   c.
   d.
   e.

2. Compare and contrast:

   ▼ mutations, selective breeding and genetic engineering

   ▼ DNA replication and protein synthesis

   ▼ transcription and translation

3a. What is the difference between a DNA sequence of codons and an RNA sequence of codons?

b. Why do we say that DNA determines the structural arrangement of proteins?

4. Suppose an individual has a nutrient deficiency due to poor diet and is missing a particular amino acid. Which process of protein synthesis would be more affected? Why?

5a. What is the relationship of the "active" and "dummy" strands of DNA? What do they look like?

b. Would there be a problem if the dummy strand were used to make a protein? Why?

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How Genes Make Proteins

STUDENT PAGE 5

6a. Given below are some tRNA anticodons/amino acid relationships and a stretch of imaginary DNA. Fill in the missing boxes in the chart below by writing the correct mRNA codons, tRNA anticodons and amino acids.

Use the following tRNA/amino acid relationships:	GGC pro	UUA asn	CAG val	CUC glu	GAU leu	AGG ser	CCG gly

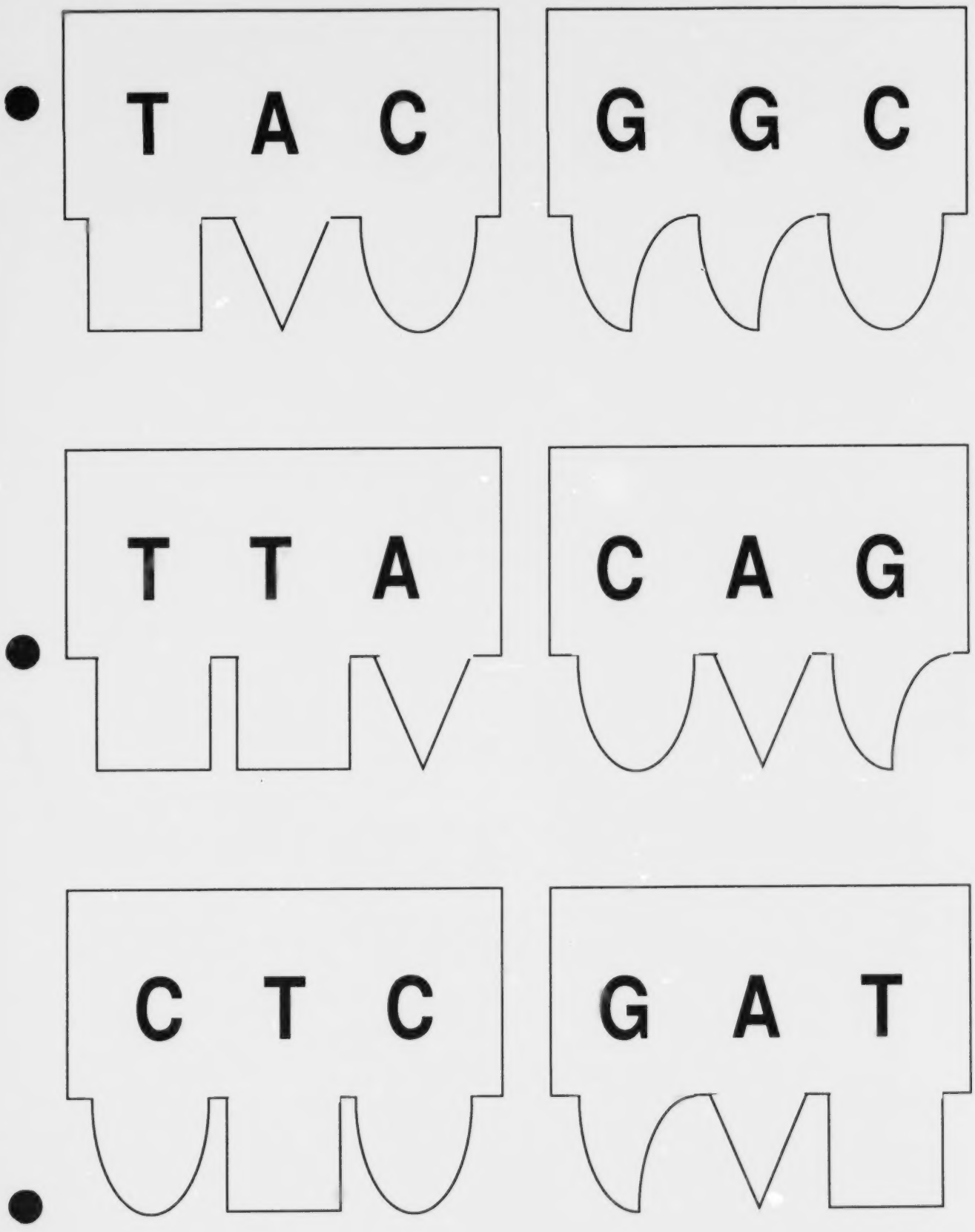
DNA:	TAC	AGG	GGC	CTC	TTA	CAG	CTC	GAT	AGG	CCG	GAT	ATC
mRNA												
tRNA												
Amino Acid	*met* **start**											**stop**

b. What are the similarities between the DNA sequence and the tRNA sequence?

7. A new and exciting branch of biotechnology is called protein engineering. To engineer proteins, molecular biologists work backward through the protein synthesis process. They first determine the exact sequence of the polypeptides they want, and then create a DNA sequence to produce it. Use the rules of transcription and translation to "engineer" the peptide sequence below. Fill in the rows for tRNA anticodons, mRNA codons and DNA.

Use the following tRNA/amino acid relationships:	GGC pro	UUA asn	CAG val	CUC glu	GAU leu	AGG ser	CCG gly

DNA												
mRNA												
tRNA												
Amino Acid	met **start**	leu	val	pro	gly	asn	ser	glu	glu	pro	val	**stop**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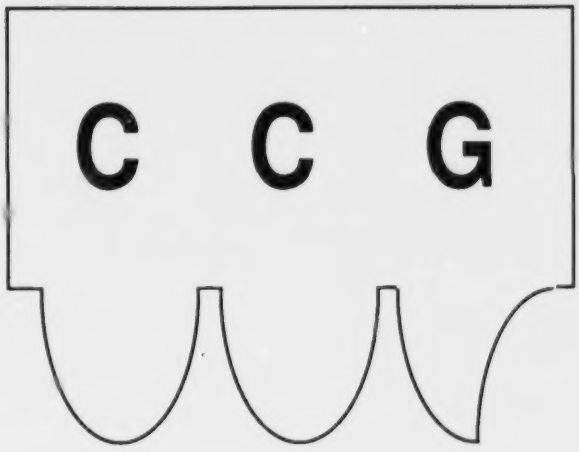
G G C
T T A
C A G
C T C
G A T

DNA DNA

DNA DNA

DNA DNA

A G G

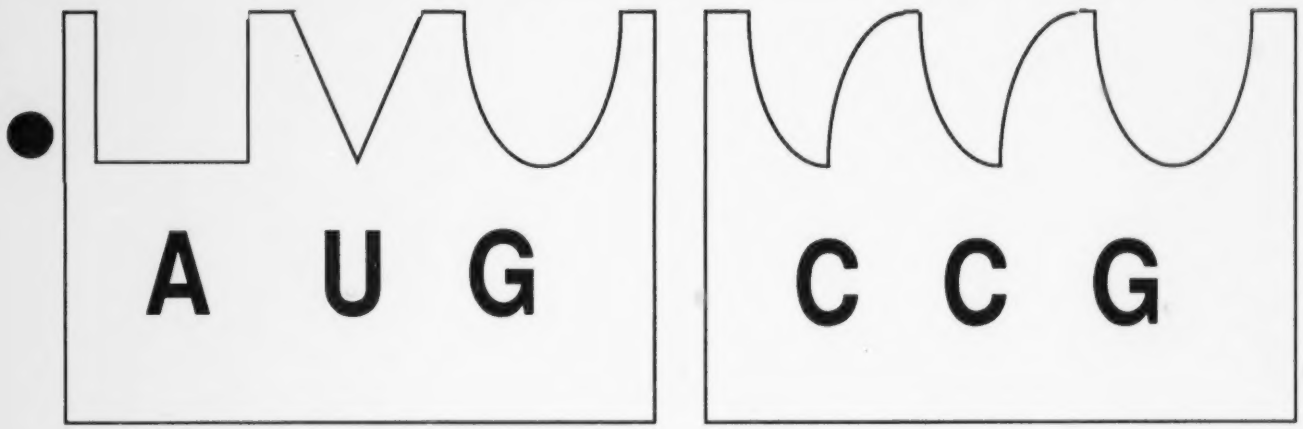

C C G

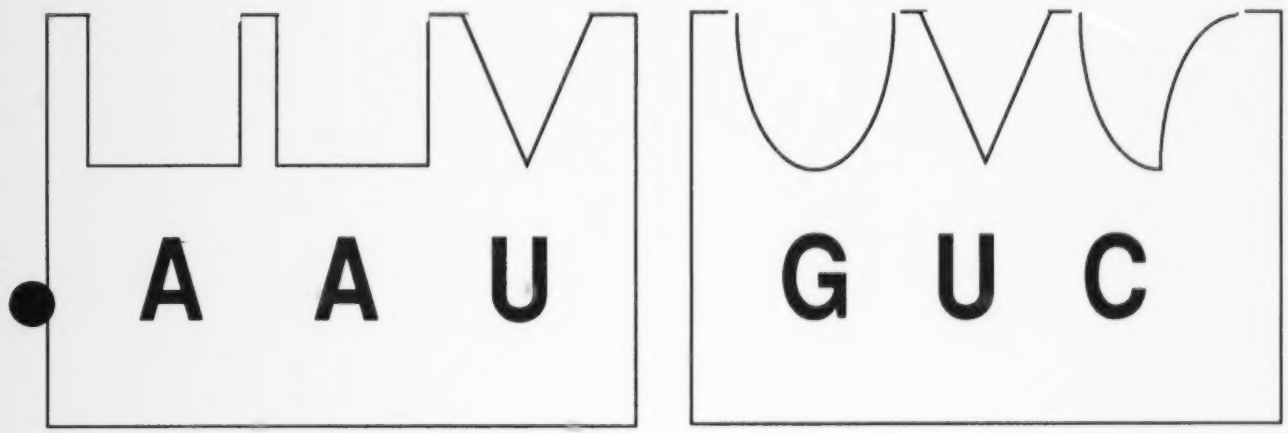
A T C

DNA

DNA

DNA


A U G
C C G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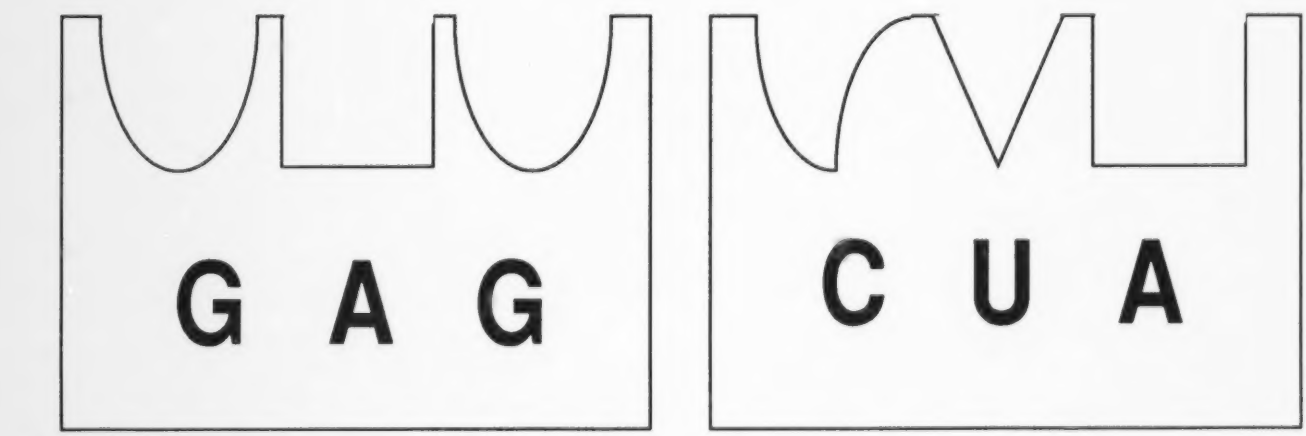
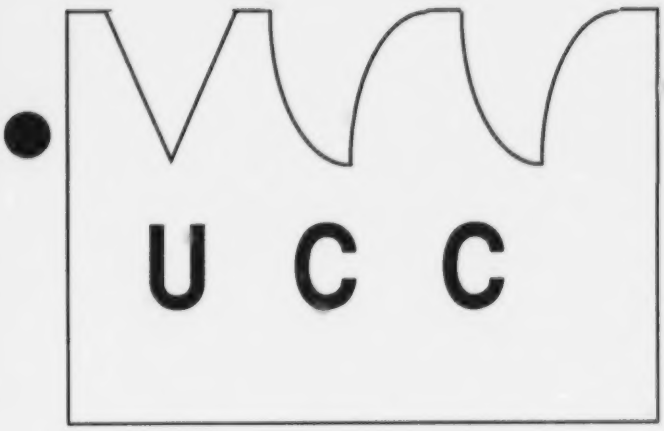
G U C

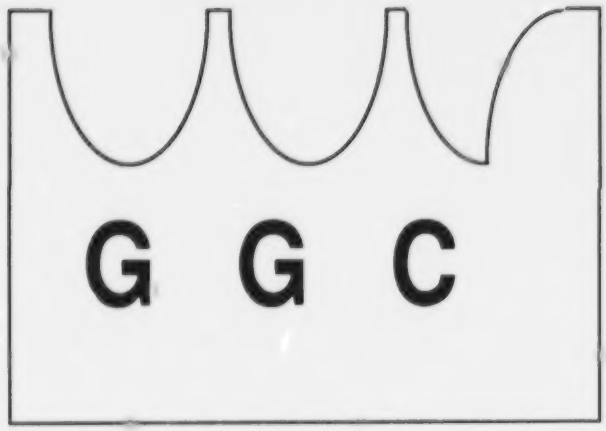

G A G
C U A

mRNA mRNA

mRNA mRNA

mRNA mRNA


U C C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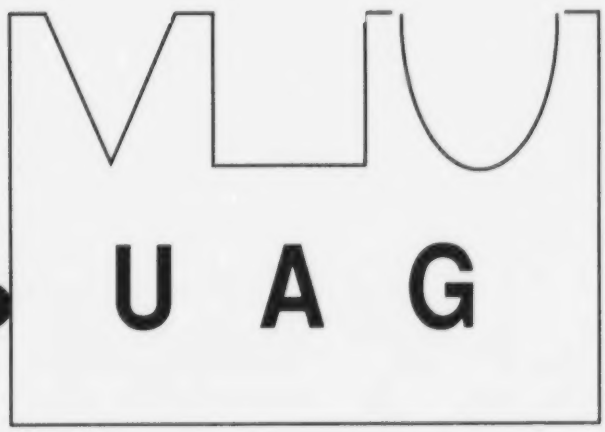
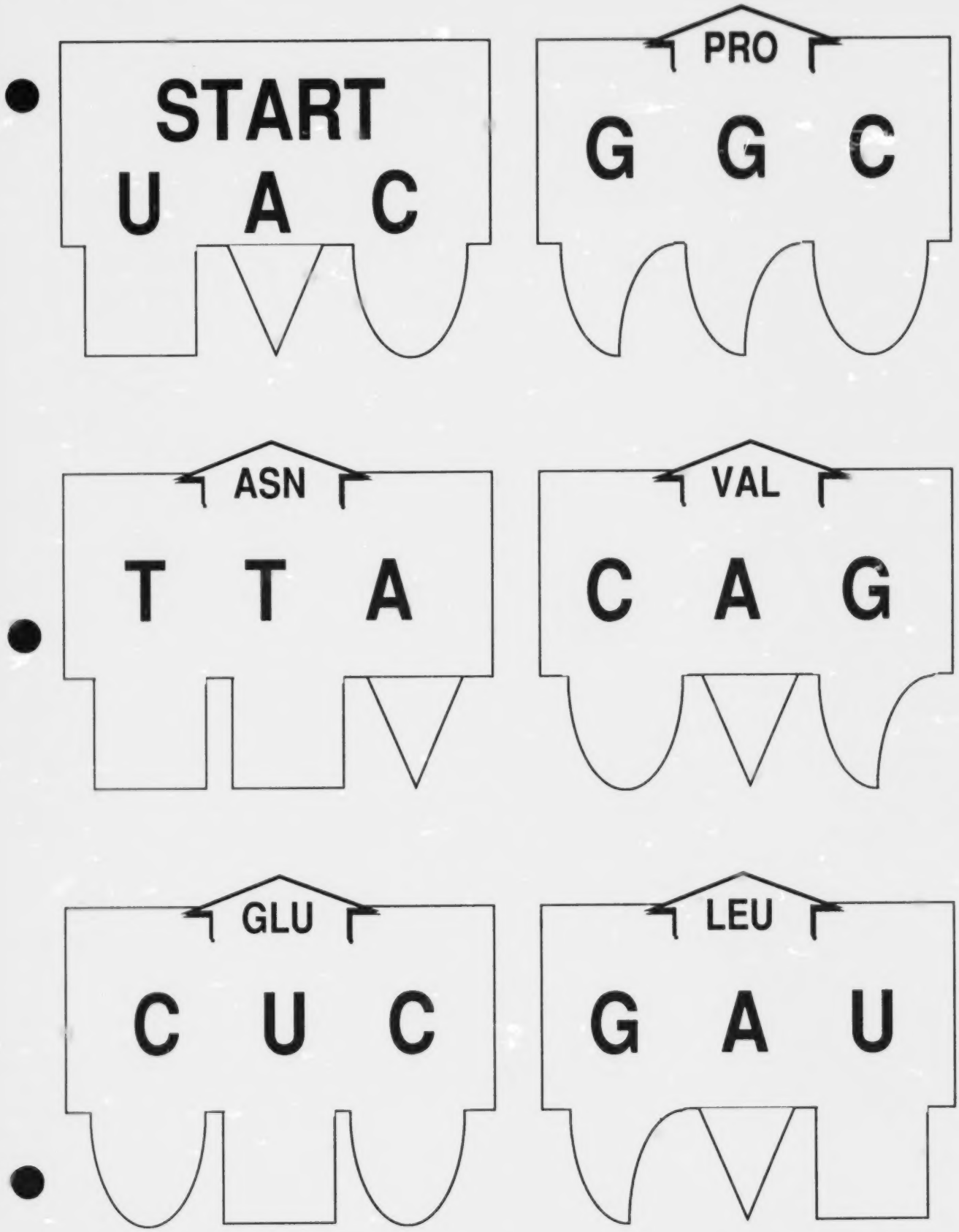

U A G

mRNA

mRNA

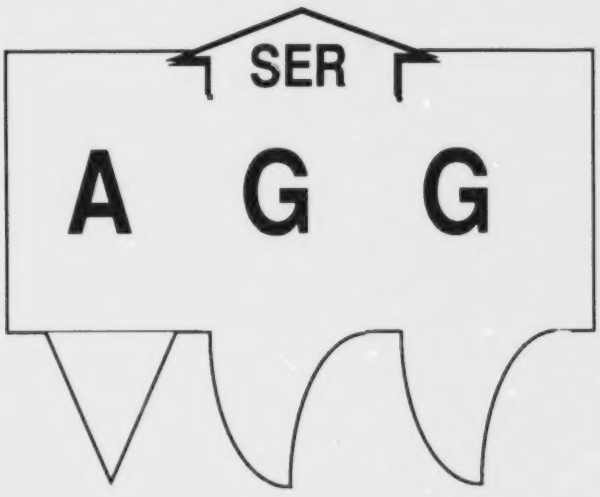
mRNA

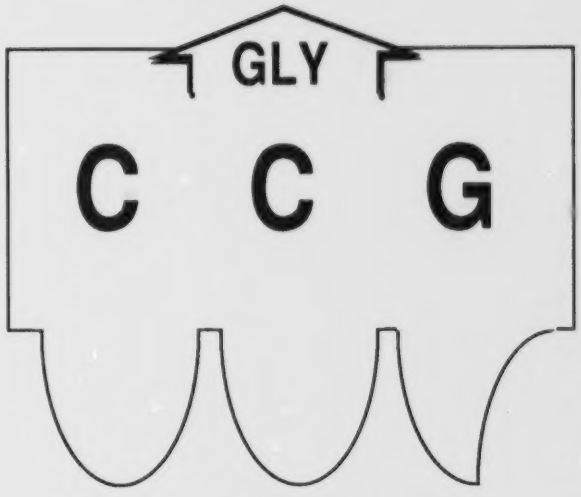

START
U A C
PRO
G G C
ASN
T T A
VAL
C A G
GLU
C U C
LEU
G A U

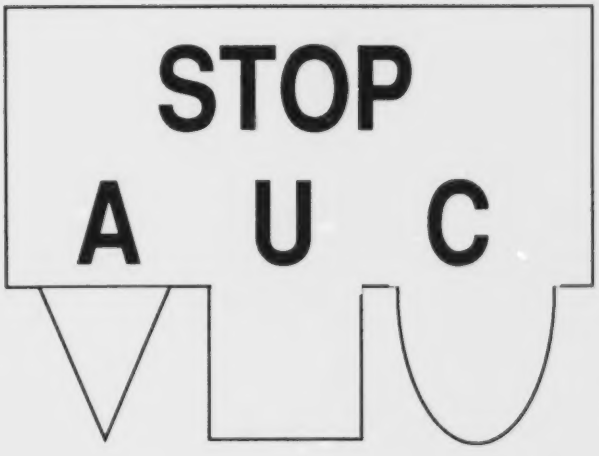
tRNA tRNA

tRNA tRNA

tRNA tRNA


SER
A G G


GLY
C C G


STOP
A U C

tRNA

tRNA

tRNA

PROLINE

ASPARAGINE

VALINE

GLUTAMIC ACID

LEUCINE

SERINE

GLYCINE

Amino Acid

Amino Acid

Amino Acid

Amino Acid

Amino Acid

Amino Acid

Amino Acid

The History of DNA: A Timeline

Ability level .....Grade 7 life science through Grade 12 AP biology
Class periods required .....1 – 3

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Introduction

Most of the knowledge about genetics came from discoveries made by relatively few scientists working over the past 150 years. Beginning with Darwin, this lesson allows students to examine some of the questions that scientists asked to explain basic genetics. The general form and sequence of these questions is taken from *DNA Science*, and the questions can be studied there in greater detail.

Few biology textbooks combine all of these questions together to show how the study of genetics evolved. This lesson will allow teachers to extend their coverage of genetics history past Watson and Crick. In addition, the lesson also offers to teachers the option of giving students a chance to work with the computer in the science classroom.

Objectives

At the end of this activity, students should be able to:

1. Describe some of the historical events and evidence that have contributed to our knowledge of DNA, and the relationship of DNA history to modern biotechnology.
2. Know the contributions of the following researchers: Darwin, Mendel, Sutton, Morgan, Beadle and Tatum, Griffiths, Avery, Hershey and Chase, Chargaff, Watson and Crick, Wilkins and Franklin, Berg, Cohen and Boyer.
3. Recognize and understand the following words: **natural selection, genotype, phenotype, chromosomes, genes, heredity, transformation, recombinant DNA, genetic engineering.**

Materials

For entire class

- overhead projector
- overhead transparencies
- illustrations, diagrams and blackline masters

Optional: Timeliner manual and program

For each student

- graph paper

Teacher Preparation

1. Read through the student pages and assemble illustrations or diagrams on the history of genetics that can be used as handouts or as overhead transparencies. Chapters 2 and 3 in *DNA Science* (see Resources section) contain some good materials. Overhead transparencies can also be made from the blackline masters that are included with this lesson. In addition, make the following overhead projections or slides:
   - differences in Galapagos Islands finch beaks (Darwin)
   - Punnett squares (Mendel)
   - chromosomes and genes (Sutton and Morgan)
2. If possible, obtain a Timeliner program and run through it yourself.

Teaching Tips

1. You may choose to add a relevant touch to this lesson by asking the students to add significant historical events (birthdays, popular music, U.S. presidents in office, etc.) to their DNA Timeline. Adding historic events to the timeline will give students an idea as to what was occurring in the world as our knowledge of genetics was evolving.
2. You may want to provide students with photocopies of the *Sourcebook*'s glossary.
3. Use videotapes, books or articles on historical figures related to DNA science as support materials, if you wish.

Procedure

1. The information in the student pages can be delivered as a lecture, a reading assignment or a series of student reports. Under each subheading is a brief historical sketch that will help shape class discussion or student summaries. Encourage students to volunteer how they would set up some of the experiments that they will read about. Also point out to students that the answer to one question provides a foundation for the next question. For example, Mendel's model of heredity was based on hereditary "factors." Mendel's model paved the way for later scientists to identify those factors, eventually to learn how heredity functions at the level of molecules.
2. After you have discussed the topic or had students complete their own summaries (individually or in small groups), instruct students to prepare a timeline. This activity provides a good opportunity for students to practice how to set up the axis of a graph (divide # of blocks by # of years covered by DNA history = 150 years).
3. Students should plot the major events covered in the student pages. Discuss with students the # of years DNA history spans, gaps in significant events, questions on vocabulary, relative "newness" of DNA history compared to the age of the earth (3-4 billion years old!), etc.

Adapted from Micklos, D.A. & Freyer, G.A. (1990). *DNA science: A first course in recombinant DNA technology*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory & Burlington, NC: Carolina Biological Supply Company. 477 pp.

The History of DNA: A Timeline

4. **Optional:** It is highly recommended that teachers use the Timeliner program so students can plot their data.

Answers to Student Questions

1. *It is more effective to have the students brainstorm this one!*
2. *Library research: good opportunity to enhance students' library skills (card catalog, Newsbank, Reader's Guide, etc.)*
3. *Use the Timelinerprogram for this one.*

Resources

Audiovisuals

The Biological Revolution: 100 Years of Science at Cold Spring Harbor Laboratory. Traces 100 years of the development of modern genetics and society. Available from: Cabisco Biotechnology, 2700 York Rd., Burlington, NC 27215; 800-334-5551 or 800-632-1231 (NC only).

Printed materials

Micklos, D.A. & Freyer, G.A. (1990). DNA Science: A First Course in Recombinant DNA Technology. Cold Spring Harbor Laboratory & Carolina Biological Supply Company. 477 pp. Available from: Cabisco Biotechnology, 2700 York Rd., Burlington, NC 27215; 800-334-5551 or 800-632-1231 (NC only).

Computer software

Timeliner (90 Sherman St., Cambridge, MA 02140; 800-342-0236), designed by Thomas F.F. Snyder & David Kaemmer (1986). Tom Snyder Productions.

The History of DNA: A Timeline

STUDENT PAGE 1

Introduction

Long ago, biologists began asking how traits were passed from one generation to the next. It was eventually determined that DNA was the molecule of heredity. Also important was the discovery that DNA was the universal molecule of heredity among organisms (excluding some viruses). Could the DNA of one organism be transferred and expressed in another organism? The answer to this question spawned the age of genetic engineering that began in the early 1970s. This lesson traces the history of our understanding of heredity, highlighting the crucial discoveries that now enable us to genetically engineer organisms.

Biologists study model systems to test their hypotheses about the nature of life. Biological model systems are simplified biological environments that can be used to explain larger biological events. The purpose of model systems is to avoid the clutter and confusion of larger and more complex biological systems. For Darwin, the finch populations in the limited environment of the Galapagos Islands provided a model system to help explain the origin of plant and animal species. Mendel used pea plants to explain the mechanisms of heredity for all organisms. Different scientists have asked questions about heredity that focus on different levels of biological organization (see Figure 1).

Most of the knowledge about heredity came from discoveries made by relatively few scientists working over the past 150 years. Beginning with Darwin, this lesson will help you examine some of the questions that scientists asked to explain the genetic basis of heredity.

Table 1. A History of Questions

How do we account for the diversity of species?	Charles Darwin
How are traits passed from generation to generation?	Gregor Mendel
Where are the units of heredity ("factors") located?	Walter Sutton Thomas H. Morgan
What do genes do?	George Beadle Edward Tatum
What molecule carries genetic information?	Frederick Griffiths Oswald Avery
What is the structure of the DNA molecule?	James Watson Francis Crick Maurice Wilkins Rosalind Franklin Erwin Chargaff
Can DNA from different organisms be recombined?	Paul Berg Stanley Cohen Herbert Boyer
How can DNA be altered to produce products?	Today's scientists

Procedure

Use the information to plot the history of DNA on a timeline. You will need to attach many pieces of graph paper together to make your line big enough. To set up the axis, divide the number of blocks by the number of years covered by DNA history = 150 years.

The History of DNA: A Timeline

STUDENT PAGE 2

DNA History:

1. *How do we account for the diversity of species?*

1859

Charles Darwin published *On the Origin of Species*. He hypothesized that animal and plant populations become adapted over time to best exploit the environment. As he traveled in the Galapagos Islands, he observed how the finches' beaks on each island were adapted to their food sources. Darwin theorized that the creatures best suited to their environment survive and reproduce. Because the better adapted organisms leave more offspring, the genetic make-up of the population changes to include more copies of their genes. This differential survival and reproduction of individuals is known as natural selection. As populations become increasingly different genetically, new species eventually are formed.

Species evolve through natural selection, but. . .

2. *How are traits passed from generation to generation?*

1865

Gregor Mendel published *Experiments in Plant Hybridization*. Mendel's question was different in scale from Darwin's. Darwin investigated how entire species originate, but Mendel investigated the mechanism for passing on specific traits within a species. Mendel guessed that invisible internal units of information account for observable traits, and that these "factors" are passed from one generation to the next. Your teacher will show you some examples of Punnett squares to illustrate this point.

Mendel's theory led to the current concept that the visible phenotype of an organism is related to an invisible genotype within the organism. Using pea plants as a **model system**, he showed how phenotypic characteristics, such as the color of flowers or the height of plants, are inherited from one generation to the next. The manner in which different traits appeared or disappeared in succeeding generations awakened Mendel to facts that are of fundamental importance in our understanding of heredity: Phenotypic traits are controlled by discrete hereditary "factors"; these factors occur in pairs; these paired factors separate, or **segregate** from each other when the parent organism forms sex cells. By following multiple traits, Mendel also hypothesized that different traits, and, thus, different "factors" could be passed on and expressed independently of each other. That is, in the formation of sex cells, paired factors sort themselves independently of each other (**independent assortment**). He also hypothesized that the factors occurred in dominant and/or recessive forms. Mendel's work remained unnoticed until 1900 when Hugo de Vries and Carl Correns published research corroborating Mendel's mechanism of heredity.

Certain 'factors' exist within organisms that are passed from one generation to the next, but. . .

3. *Where are these 'factors' located?*

1902

Walter Sutton suggested that Mendel's "factors" are located on chromosomes. After observing chromosomal movements during meiosis, Sutton developed the chromosomal theory of heredity. Sutton noticed that chromosomes occurred as pairs, and that gametes receive only one chromosome from each pair when they form during meiosis. This corroborated Mendel's theory that the genetic "factors" were segregated. Walter Sutton gave Mendel's "factors" the name we use today: "genes." The first direct evidence for the chromosomal theory of inheritance came in 1905 when Edmund Wilson and Nellie Stevens proposed the idea that separate X and Y chromosomes determine sex. They showed that a single Y chromosome determines maleness, and two copies of the X chromosome determine femaleness.

1910

Thomas H. Morgan proved that genes are carried on chromosomes, establishing the basis of modern genetics. With his co-workers at Columbia University, he pinpointed the location of various fruit fly genes on chromosomes. Morgan's group also demonstrated the existence of sex-linked genes, and over the next 10 years expanded the idea to other trait linkages. They used crossing-over to measure the locations of genes. Morgan and his group established the use of fruit flies to study heredity. Using the frequency of crossing over, Alfred Sturtevant calculated the distances between genes and made the first gene maps of chromosomes.

Genes are located on chromosomes and are the units of heredity, but. . .

The History of DNA: A Timeline

STUDENT PAGE 3

4. *What do genes do?*

1927
Herman Muller showed that exposing fruit flies to X-rays increased the mutation rate by about 1,500 times. This did not answer questions about how genes function, but it provided researchers with a way to induce mutations. Inducing mutations would prove to be an important tool for finding out what genes do.

1941
George Beadle and Edward Tatum presented the "one gene, one enzyme" hypothesis: Each gene is translated into an enzyme to perform tasks within an organism. Beadle and Tatum found a mold (*Neurospora*) that synthesized all the chemicals it needed from a simple nutrient medium. They examined X-ray-damaged mold specimens that would *not* grow on the simple medium, but *would* grow if they added a certain vitamin. They hypothesized that the X-rays had damaged the gene that is translated into an enzyme that is involved in the synthesis of the vitamin (Figure 2). Although Beadle and Tatum showed that genes synthesize proteins, most biologists still believed that genes *were* proteins.

Genes are responsible for synthesizing proteins, but. . .

5. *What molecule carries genetic information?*

1869
A chemist named Johann Meischer isolated DNA from the nuclei of white blood cells. Mendel's theory of "factors" was not yet known, and Meischer was not investigating heredity. Instead, he was trying to identify the chemicals in cells. Although Meischer knew nothing about the structure of DNA or its role in biology, his discovery paved the way for others. DNA was known long before scientists began to suspect its function.

1928
Fredrick Griffiths noticed that a rough kind of bacterium changed to a smooth type when an unknown**"transforming principle"** from the smooth type was present (Figure 3). Sixteen years later, Oswald Avery noticed Griffiths' results.

1944
Oswald Avery theorized that DNA, not protein, was Griffiths' "transforming principle." Avery and his group performed a transformation experiment with Griffith's bacterium. Using purified extract of the smooth type's protein coat and purified extract of the smooth type's interior, Avery separately mixed each with the live rough type of bacterium. The rough type was transformed to smooth only by the purified interior of the smooth type. Therefore, Avery reasoned that the "transforming principle" must be in the bacteria's interior.

Avery's team separately treated the purified extracts of the smooth bacteria with three enzymes: one to break down protein, one to break down RNA and one to break down DNA. The protein- and RNA-digesting enzymes did not diminish the transforming power of the smooth bacteria extract. However, when the DNA-digesting enzyme destroyed the DNA, all transformation stopped. (An illustration of Avery's experiment can be found on page 18 of *DNA Science.*) This research clearly showed that DNA was the transforming principle, but it gained little attention because scientists believed that DNA was too simple a molecule to contain all of the genetic information for an organism. Most scientists believed that only proteins with their many amino acid building blocks were complex enough to express all of the genetic combinations. After all, DNA has only four coding units.

1945
Max Delbruck and Salvador Luria organized a course at Cold Spring Harbor Labs, located on New York's Long Island, to study a type of bacterial virus that consists of a protein coat containing DNA. These bacteriophages were interesting because they provided a very simple model system for studying how genetic information is transferred to host bacterial cells. Delbruck and Luria's course attracted many scientists to Cold Spring Harbor, which soon became a center for new ideas about explaining heredity at the cellular and molecular levels.

1952
Alfred Hershey and Martha Chase performed the famous "blender experiments" using phages (Figure 4). They theorized that if they could separately "tag" both the DNA and proteins of the phages, then the DNA and proteins could be followed through the phages' replication process. A new discovery, radioactive labeling, supplied the process with his "tag."

The History of DNA: A Timeline

STUDENT PAGE 4

Hershey and Chase used radioactivity to tag the proteins of one group of phages and the DNA in another group of phages. They then allowed both groups of phages to infect different populations of bacteria. After the phages had injected their genetic material, the phages were knocked off of the bacterial cells by placing the cultures in Waring blenders. The phages and bacteria of both populations were then checked for radioactivity. It was determined that only those phages whose protein coats had been tagged were still radioactive.

Next, Hershey and Chase allowed the phages to reproduce inside the bacteria, producing daughter phages. The daughter phages were then checked for radioactivity. The results showed that the daughters of the DNA-tagged phages were radioactive, but the daughters of the protein-tagged phages were not. Radioactive DNA had entered the bacterial cells and replicated itself. They concluded that the instructions for assembling new phages had to be carried by DNA and not protein.

DNA is the molecule of heredity, but. . .

6. *What is the structure of the DNA molecule?*

1950
Erwin Chargaff determined there is always a ratio of 1:1 adenine to thymine in DNA of many different creatures. Chargaff's information would be used three years later to determine the structure of the DNA molecule.

1953
James Watson and Francis Crick examined several clues to determine the structure of DNA. They knew that the constituent parts of DNA were phosphoric acid, deoxyribose, adenine, guanine, thymine and cytosine. From Rosalind Franklin's and Maurice Wilkin's X-ray crystallography, Watson and Crick had clues about the width of the molecule and the atomic distance between subunits, and they knew there was a constant 1:1 ratio of adenine to thymine. Using paper and metal models, they conceived a structure that accounted for all the clues and had the capacity to store genetic information. The molecule could unzip along the weak hydrogen bonds and attract complements identical to the original pairing. Adenine could bond only with thymine and guanine only with cytosine.

1957
Francis Crick and George Gamov worked out the "central dogma" explaining how DNA functions to make protein (Figure 5). The central dogma stated that DNA *transcribes* into *messenger* RNA, and messenger RNA translates its message into protein. This dogma has been amended since 1957.

1966
Marshall Nirenberg and Severo Ochoa discovered that specific nucleotide sequences in groups of three determine each of 20 amino acids.

DNA structure allows for DNA replication and protein synthesis, but. . .

7. *Can DNA from different organisms be combined in the lab?*

1972
Paul Berg cut sections of viral DNA and bacterial DNA with the same restriction enzyme. Since the structure of DNA is universal between organisms, the ends of the DNA were compatible. He successfully spliced the viral DNA to the bacterial DNA.

1973
Stanley Cohen and Herbert Boyer created a plasmid with dual antibiotic resistance by patching together two DNA fragments with different specific antibiotic resistance. Cohen's group placed this recombinant DNA molecule in bacteria, thus producing the first recombinant DNA organism.

8. *How can DNA be altered to produce products?*

1977
The first practical application of genetic engineering was the expression of the gene for the human hormone somatostatin in reproducing bacterial cells. This indicated the potential for cloning valuable and useful genes in bacteria. Sixteen bills were introduced in Congress to regulate recombinant DNA research. The bills called for the development of safe bacteria and plasmids that could not escape from the lab. None of the bills passed, but the National Institutes of Health released guidelines restricting many categories of recombinant DNA research.

The History of DNA: A Timeline

STUDENT PAGE 5

1978
Harvard researchers used genetic engineering techniques to reproduce rat insulin. Genentech, a biotechnology company, produced human insulin. Stanford University scientists successfully transplanted a mammalian gene. The discoverers of restriction enzymes received the Nobel Prize in medicine.

1979
Genentech produced a human growth hormone and two kinds of interferon. DNA from malignant cells transformed a strain of cultured mouse cells. The resulting malignant cells were a powerful new tool for analyzing cancer genes.

1980
The U.S. Supreme Court decided that man-made microbes could be patented.

1988
The first living mammal was patented. Harvard University received the patent on a line of genetically engineered laboratory mice used in cancer study.

Questions

1. Polymer (plastics) chemistry (1920–), petrochemicals (1930–), atomic physics (1940–) and computers (1950–) are some of the recent revolutions in science. Compare and contrast these revolutions to the age of modern biotechnology (1970–).

2. Most of the scientists in this lesson explained their research to their peers by delivering or publishing a paper or book. Present a one- or two-page abstract of a DNA discovery whether covered in this lesson or not. The research phase will take from two to five days.

3. The industries of agriculture, medicine, food processing and bioremediation all are benefiting from the advancements in molecular biology. Research the major events of one of the above industries and make a timeline like you did with DNA history. Indicate on the timeline the events that are related to advances in modern molecular biology.

Figure 1

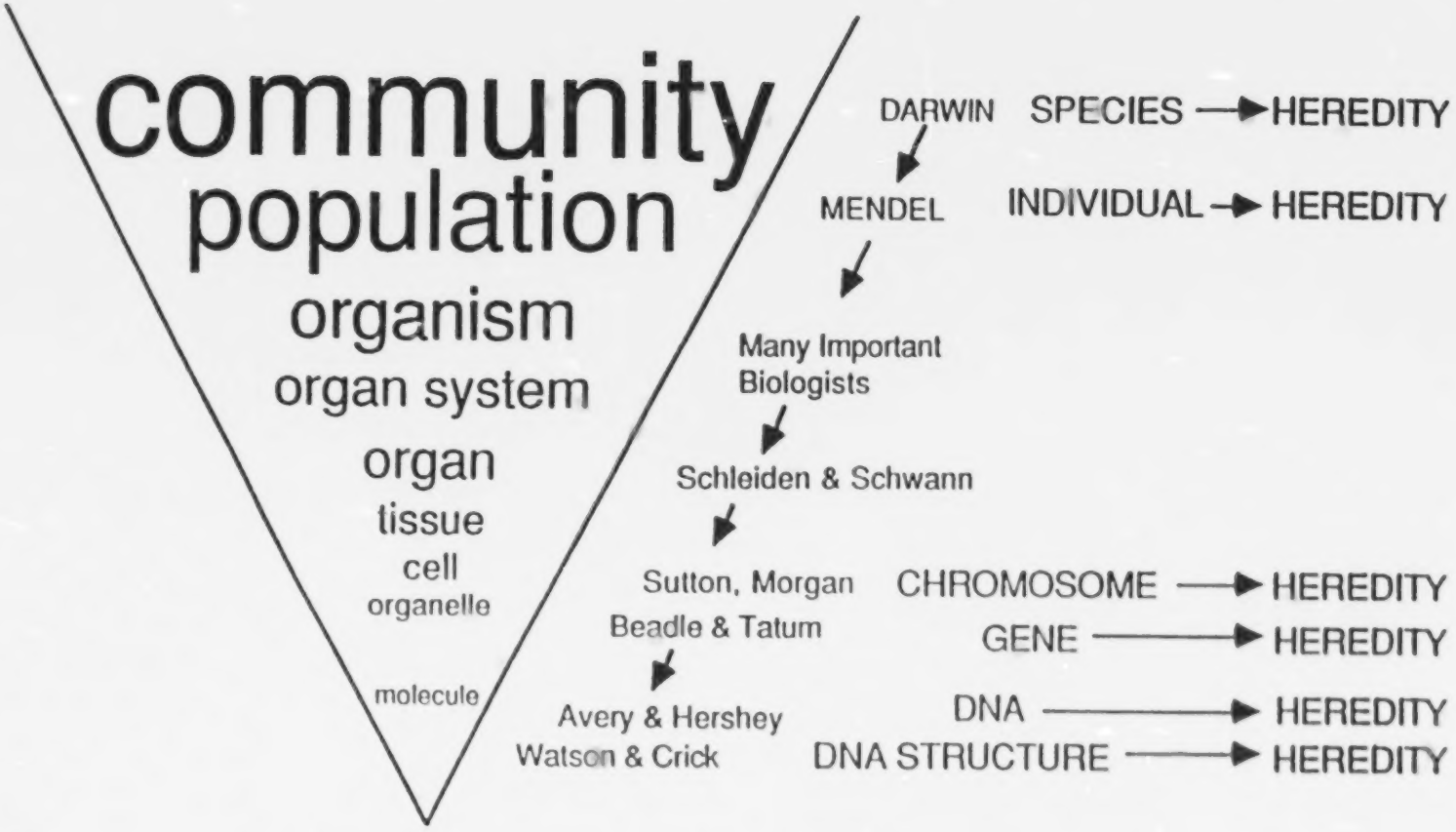
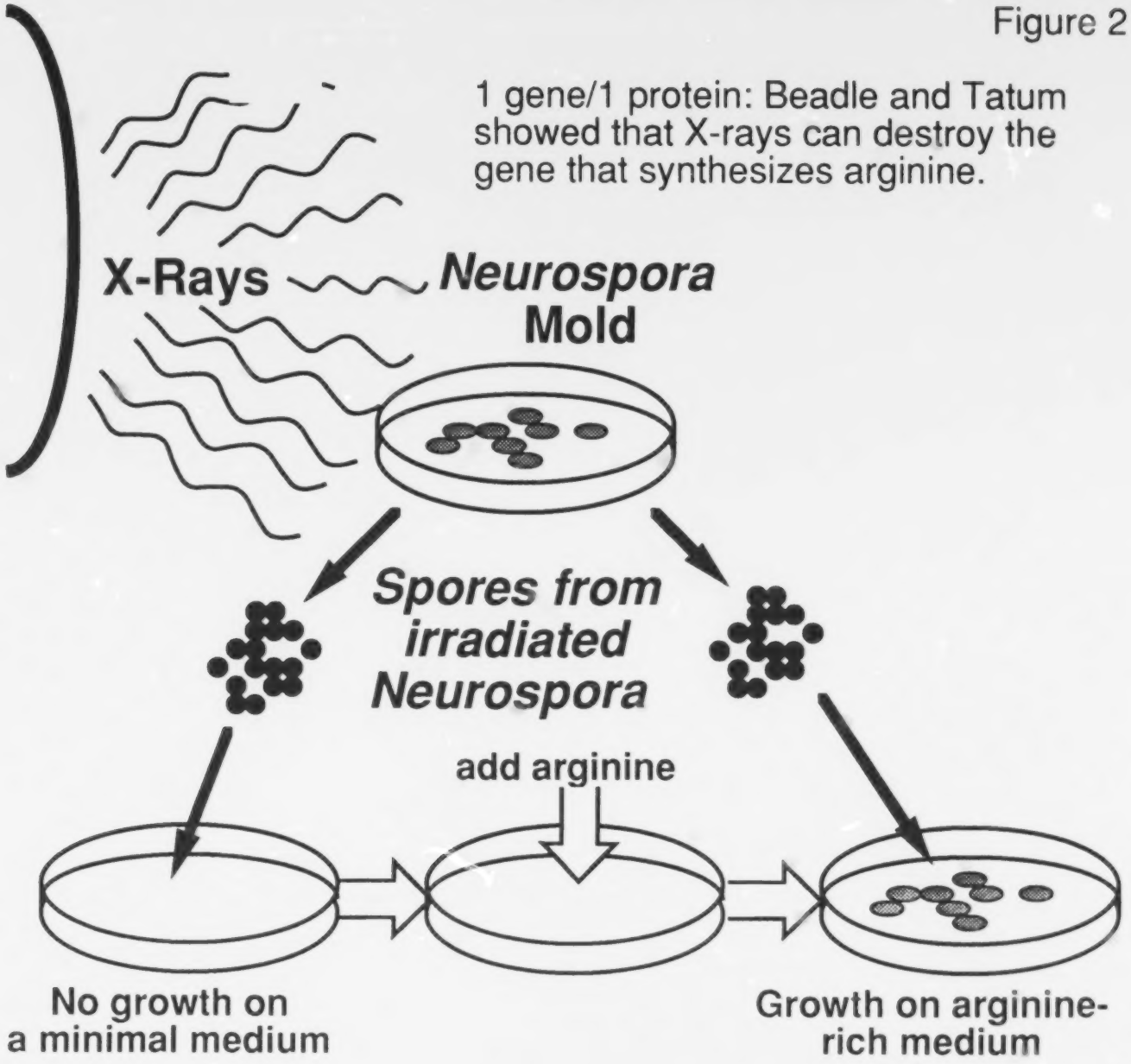

community
population
organism
organ system
organ
tissue
cell
organelle
molecule
DARWIN SPECIES → HEREDITY
MENDEL INDIVIDUAL → HEREDITY
Many Important Biologists
Schleiden & Schwann
Sutton, Morgan CHROMOSOME → HEREDITY
Beadle & Tatum GENE → HEREDITY
Avery & Hershey DNA → HEREDITY
Watson & Crick DNA STRUCTURE → HEREDITY
Cohen, Berg, & Boyer rDNA → HEREDITY

Figure 2

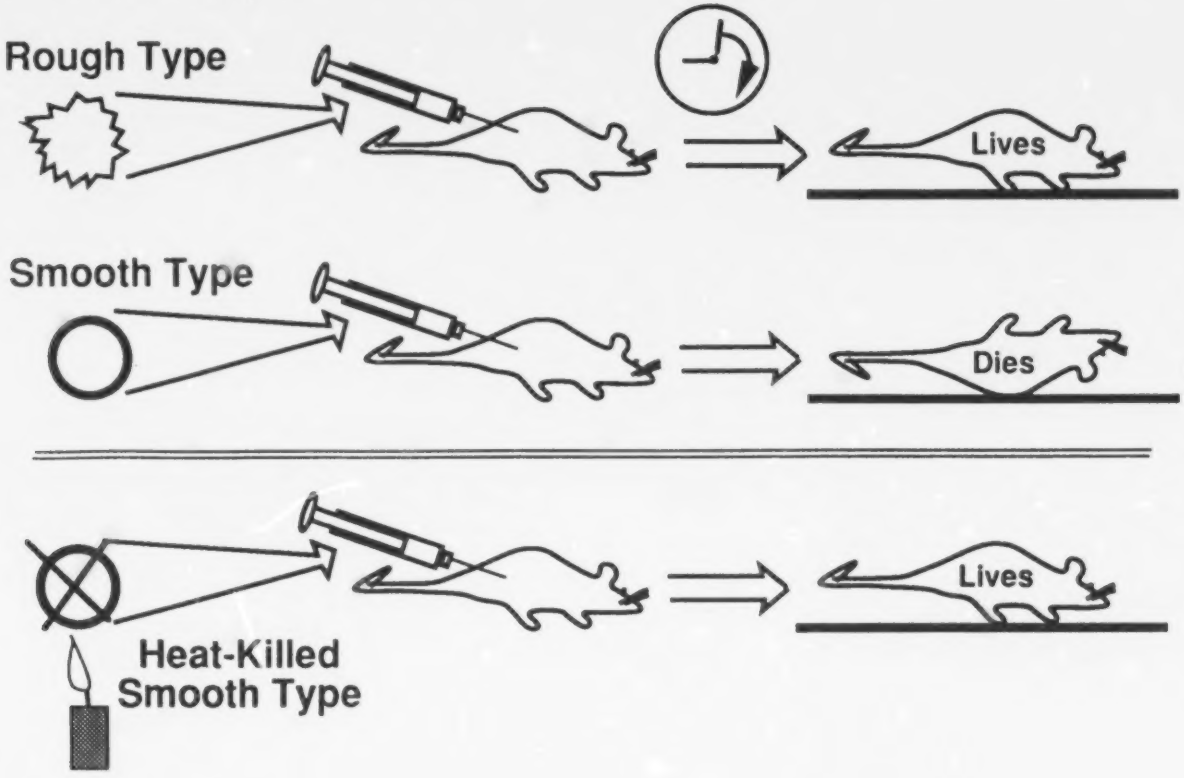
1 gene/1 protein: Beadle and Tatum showed that X-rays can destroy the gene that synthesizes arginine.



Griffith's Experiment:

A "transforming principle" exists in smooth pneumonia bacteria.

Figure 3



Hershey's Blender Experiment:

Figure 4

Phages descended from parents with radioactive DNA also have radioactive DNA.
Radioactive protein is not passed to offspring

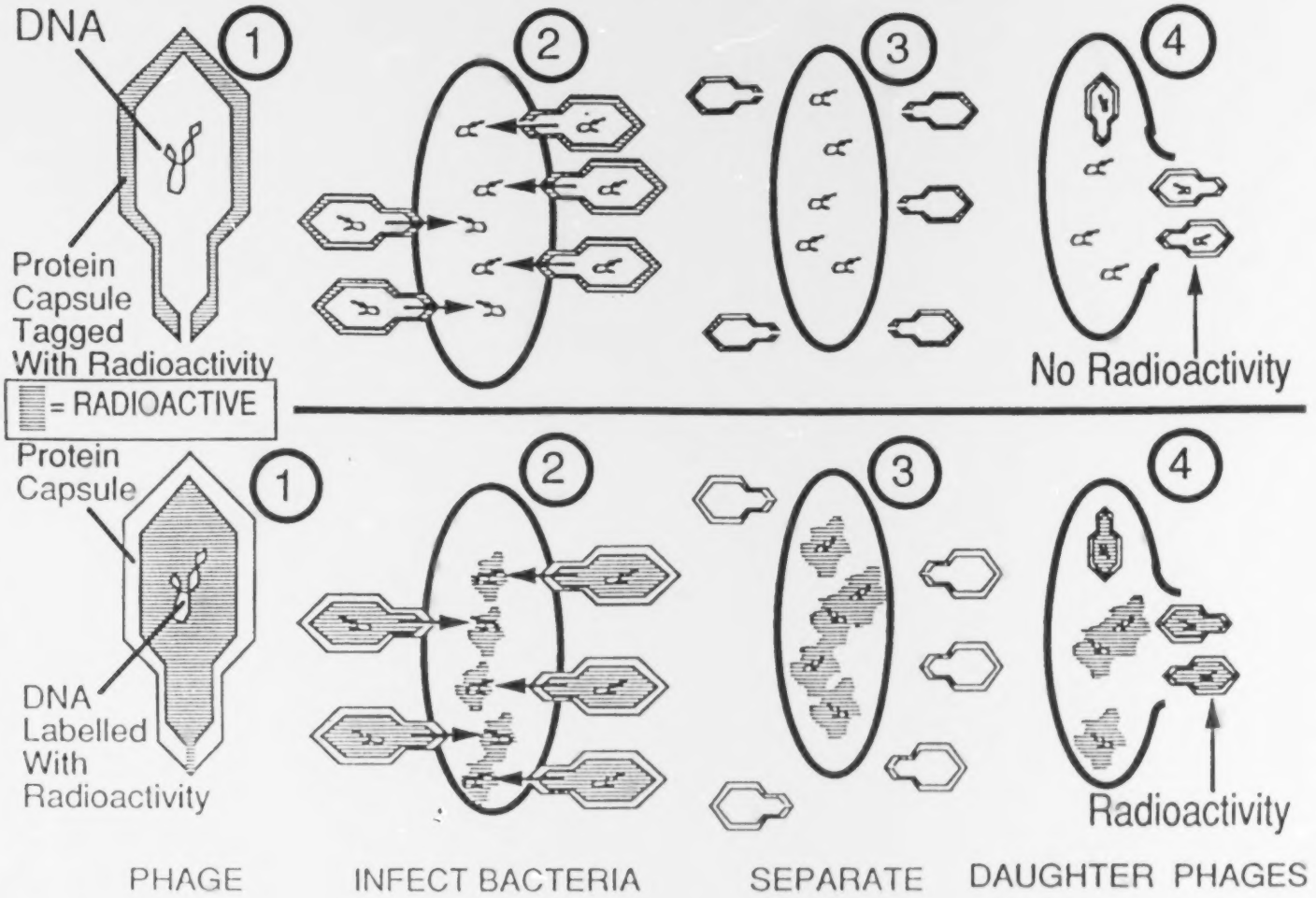
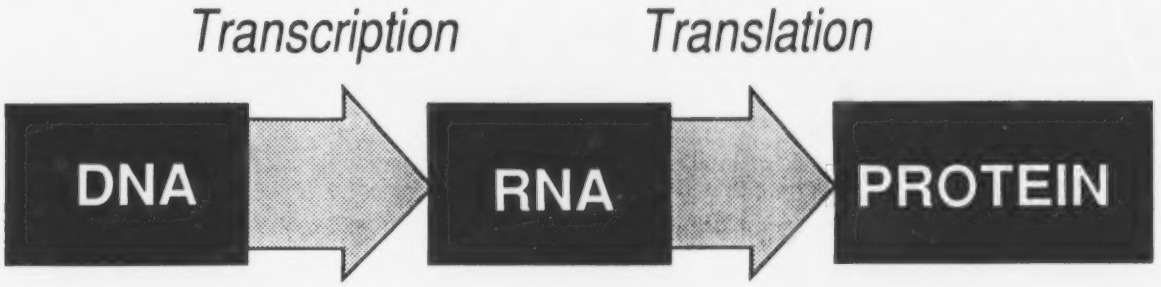


Figure 5



Francis Crick's "Central Dogma"

Madam, I'm Adam

Ability level ..... Grades 6-10
Class periods required 1

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Burtonsville, MD 20866
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Introduction

In this lesson students read background information to gain an understanding of restriction enzymes and their recognition sites. Palindromes are used to illustrate DNA sequences. At the end of the reading, students work through a series of exercises and questions.

This lesson can be used as a follow up to a lesson on DNA structure or as an introduction to restriction enzymes.

Objectives

At the end of this lesson, students should:

1. Understand the function and application of restriction enzymes in biotechnology.
2. Recognize a palindrome and understand its significance as a recognition site.
3. Be able to explain the role of restriction enzymes in genetic engineering.
4. Recognize and understand the following words: **palindrome, genetic engineering, restriction enzymes, restriction sites**

Materials

Each student will need a copy of the reading and worksheets.

Teacher Preparation

Photocopy the student reading and worksheets.

Procedure

1. Students read "Background Reading."
2. Students do the exercises and questions on the Worksheet.

Answers to Student Questions

1. *Palindromes are recognition sites for restriction enzymes. Knowing where the restriction sites are allows genetic engineers to use the right restriction enzyme to cut out the desired DNA.*
2. *Restriction enzymes have been isolated from bacteria. These bacteria use restriction enzymes as a defense against invading viruses and bacteria.*
3. *A gene is a segment of DNA that codes for a specific polypeptide chain made up of amino acids.*
4. *Sticky ends are staggered cuts in the double strand of DNA. This kind of a cut makes it possible for genetic engineers to insert new DNA into the strand.*
5. *If defective DNA sequences can be repaired, many genetic diseases will be eliminated.*
6. *Bioethical issues such as the ability to create a "perfect" organism. Who decides what is "perfect"? Who decides whether it is right to tamper with the genome of a species?*

Additional Activities

1. Have students repeat steps 1-4 on the worksheet, using the following examples:

BAM HI. The DNA recognition site is between G and G in the palindrome.

G ↓ GATCC

HIND III. The DNA recognition site is between A and A in the palindrome.

5' A ↓ AGCTT 3'

Hpa II. The DNA recognition site is between C and C in the palindrome.

5' C ↓ CGG 3'

Resources

Computer software

Hartley's Word Search. This program can be used to create a palindrome seek-a-word worksheet.

Madam, I'm Adam

STUDENT PAGE 1

Background Reading

An important development in biology is genetic engineering. There is a lot of excitement about this breakthrough because scientists are now able to take the genes from one organism and put them into a different organism. For instance, the gene for human insulin has been successfully inserted into bacteria. The bacteria grow rapidly, doubling their population every 20 minutes. As they grow, they release insulin into their growth medium. Scientists can extract the insulin from the growth medium for diabetics to use. This method of producing insulin is inexpensive and now provides the major source of insulin used today.

Genetic engineering is possible because of special chemicals called restriction enzymes. These enzymes are found in bacteria. Restriction enzymes are being used as chemical "scissors" to cut out genes. They are called *restriction* enzymes because they are normally used by bacteria to prevent or restrict the invasion of foreign DNA (deoxyribonucleic acid) into their cells. These enzymes cut up foreign DNA so that it can't function. After it was observed that the foreign DNA was cut at specific places by these enzymes, genetic engineering became possible.

The specific places where restriction enzymes cut are called *restriction sites*. These sites are recognized by the enzymes because they are **palindromes**. A palindrome is a word or sentence that is exactly the same forward or backward. Tot, pop and radar are simple words which are palindromes. A more complex example of a palindrome is Madam I'm Adam. If the restriction enzyme cuts only between an A and D, the sentence, "How do you do Madam, I'm Adam Apple" (minus the spaces and punctuation) would look like this:

5' HOWDOYOUDOMA⬇ DAMIMA⬇ DAMAPPLE 3'

Remember that the restriction enzyme only cuts where there is a palindrome. If there is an A D combination outside the palindrome, the enzyme won't cut it. It will also read from the 5' end to the 3' end.

There are many examples of palindromes in our language and other languages as well. One of the longest palindromes in English is: a man, a plan, a canal, Panama. Write out this palindrome without spaces or commas and "cut" it with a restriction enzyme which only cuts between A and M. How many pieces would your sentence be?

The genes that genetic engineers cut with restriction enzymes are special "words." These genetic "words" have a very small alphabet — only the letters A, T, G and C. This is because genes are made up of DNA which has four bases — **A**denine, **T**hymine, **G**uanine and **C**ystosine. On the DNA strand, A pairs with T; C pairs with G. There are also palindromes in DNA. Since there are two strands of DNA, a palindrome would look like this:

5' G A A T T C 3'
3' C T T A A G 5'

The top strand is the same forward as the bottom strand is backwards. (DNA is **antiparallel** so it will read the top strand from right to left and the bottom strand from left to right.)

If this palindrome was a recognition site for a restriction enzyme which cut only between A and G, it would look like this on a DNA strand:

5' A T T C C G⬇ A A T T C C G T C T 3'

3' T A A G G C T T A A⬆ G G C A G A 5'

The palindrome caused the restriction enzyme to make a staggered cut in the DNA. This staggered cut is called a "sticky end" because this kind of a cut makes it possible to "stick" the gene into a new strand of DNA. If the new strand of DNA was cut with the same restriction enzyme, the two pieces will join together. This process is what allows genetic engineers to cut and splice DNA.

There are many different restriction enzymes and each of them has a different recognition site. The first ever discovered was EcoRI. Its name comes from the bacterial species where it was found:

Escherichia coli **R**Y 13 and **I** for the first found in this species.

Madam, I'm Adam

STUDENT PAGE 2

Worksheet

Be sure to read all the steps before starting. Use a separate sheet of paper for your answers.

1. The recognition site for EcoRI is our old friend **GAATTC**. EcoRI cuts between G and A *within* this recognition site. Write out this recognition site as a double strand of DNA.

2. Make up a sequence of DNA using the bases A-T-C-G in any order. Use 15 bases and be sure to make two strands. Remember that A pairs with T and G pairs with C.

3. Add the recognition site of EcoRI in two different places on your DNA sequence.

4. Cut your DNA sequence by using an arrow. Be sure you have "sticky ends."

Questions

1. Why are palindromes important to genetic engineers?

2. Where do restriction enzymes come from?

3. What is a gene?

4. Write a description of a "sticky end" and explain why it is important to have this type of end when doing genetic engineering.

5. What kinds of solutions to problems in disease fighting do you think can be solved through genetic engineering?

6. What kinds of problems do you think genetic engineering might cause in the future?

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The Onion Lab:DNA Spooling

Ability level .....Grade 7 life science, Grade 10 regular & AP biology
Class periods required 1

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Introduction

Most students think of DNA as an impossibly small molecule. After all, it is inside a cell too small to be seen without a microscope and is further compressed into a nucleus inside that cell. It is not something that students normally expect to handle during a laboratory. This procedure is a quick and easy way to allow students to actually see some DNA from an organism with which they are familiar: an onion. Students first spool DNA out of an alchohol and onion mixture, then they test the DNA with diphenylamine. An optional third activity uses an agarose gel to test the DNA.

This lesson can be used after students have learned the structure and basic function of DNA.

Objectives

At the end of this activity, students should have:

1. extracted a visible mass of DNA from onion.
2. learned the basic properties of DNA.
3. confirmed the presence of the DNA by a reaction with an indicator.
4. learned to recognize and understand these words: **precipitation, phospholipid, emulsify, spooling.**

Adapted from Biology in the Laboratory *by Doris Helms.*

Materials

For teacher prep:

- 2 large beakers
- blender or mortar & pestle
- thermometer
- large funnel or colander
- cheese cloth or #6 coffee filter
- hot tap water bath (60°C)*
- ice water bath
- 2000 ml distilled water

meat tenderizer**

- 100 ml liquid dishwashing detergent***
- 1 large onion
- 20 g NaCl (or non-iodized table salt)
- 1020 ml glacial acetic acid
- 0.1 g DNA standard (Sigma D-3159)**
- 16 g diphenylamine (Fisher 0-2611)
- 1 ml acetaldehyde (Fisher 0-1004)
- 15 ml sulfuric acid

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* **Hot water:** a simple bath can be made by putting a stopper in the sink and running the hottest water from the tap. The temperature isn't too critical – just don't cook the onions with boiling water.

** **Meat tenderizer:** look in the spice section of the grocery store for any brand with the least amount of extra ingredients. The ingredient you need is *papain* – a protease that helps clean the extra proteins away from the DNA.

*** **Liquid dishwashing detergent:** any type works fine. I have used Dawn, Joy, Palmolive Green.

** **DNA standard** will keep frozen for several years.

For each lab team:

- 3 test tubes - 15x125mm*
- 1 10ml graduated clylinder or 10 ml pipet
- 1 glass rod 200mm long
- 1 plastic pasteur pipet**
- 9 ml of alcohol: isopropanol or ethanol 95%(ice cold)***
- hot plates with beakers of boiling water
- test tube holders
- pinch clamps to hold hot test tubes
- copies of the student pages

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* **Test tubes** can be any size – as long as they are big enough to hold at least 25 ml of liquid.

** **Plastic pasteur pipets:** If you fill one so it is full to half of the bulb, it will equal approximately 3.5 ml.

*** **Alcohol:** The concentration used for the precipitation is important. You need 95%, stronger than normally found on the drug store shelf. Any science supply company will have this concentration and sometimes pharmacists will have it. Put it the freezer overnight in a plastic bottle because it needs to be icy cold.

The Onion Lab:DNA Spooling

Teacher Preparation

All the amounts given can be approximations. Precision is not essential since this is a forgiving prep.

DNA spooling preparation for onion filtrate

(Steps 1 - 7 can be done a day or two ahead.)

1. Make a solution consisting of 100 ml liquid dishwashing detergent and 15.0 g NaCl. Add distilled water to make a final volume of 1000 ml.
2. Cut one large onion into pieces the right size for spaghetti sauce and put into a large beaker (500 ml).
3. Cover onions with 100 ml of the solution from step 1.
   The liquid detergent causes the cell membrane to break down and emulsifies the lipids and proteins of the cell by disrupting the polar interactions that hold the cell membrane together. The detergent forms complexes with these lipids and proteins causing them to precipitate out of the solution. NaCl enables nucleic acids to precipitate out of an alcohol solution because it shields the negative phosphate end of DNA, causing them to come closer together and coalesce. 0.3 molar concentration is required.
4. Stir mixture and let sit 15 minutes in a hot water bath at 60° C. (Any longer and the DNA begins to break down.)
   The heat treatment softens the phospholipid in the cell membrane and denatures the DNAse enzymes which, if present, would cut the DNA into small fragments so that it would not spool.
5. Cool mixture in an ice water bath for 5 minutes, stirring frequently with a spoon. This step slows breakdown of DNA.
6. Pour mixture into a blender and blend for 1 minute on low speed and 30 seconds on high speed.
   The blender breaks down the cell wall, cell membrane and nuclear membrane, allowing the release of DNA.
7. Filter the mixture through four thicknesses of cheesecloth (or a #6 coffee filter) placed in a large funnel over a large beaker. (After blending the detergent, salt and onions, the mixture will be very foamy. When you filter this, try to keep the foam from getting into the filtrate. It filters slowly, so you might want to put the whole set up in the refrigerator and let it filter overnight.)

(Steps 8 – 9 should be carried out shortly before doing the lab.)

8. Dispense 6 ml of the onion filtrate into test tubes. Prepare enough for each lab team to have a sample.
9. Mix a 6% solution of meat tenderizer (3 g/50 ml water)

DNA Testing Preparation

1. Add 0.1 g DNA to 200 ml distilled water. Add one drop glacial acetic acid. If required, heat gently to get into solution.
2. Dissolve 16 g diphenylamine in 1000 ml glacial acetic acid. Add 15 ml concentrated sulfuric acid. Store solution in a dark bottle. On the day of use, prepare a solution of fresh acetaldehyde (1 ml) in distilled water (500 ml). Add 1 ml of this solution per 100 ml diphenylamine solution.
3. 4% NaCl solution (add 2 grams of NaCl to 50 ml distilled water).
4. Have hot plates with beakers full of boiling water available around the room for this part of the lab.

Procedure

(See student pages for detailed protocol.) Students do the following:

1. Read introduction
2. DNA Spooling
3. DNA Testing
4. Answer questions

Answers to Student Questions

1. *It binds to the lipids in the cell membrane.*
2. *All vegetables have DNA. An onion is good to use because its low starch content makes it easier to obtain a clear solution for the DNA extraction process.*
3. *A. DNA is supercoiled and very compact in the cell; B. DNA is not soluble in isopropanol or ethanol); C. DNA is universally found in all living things - even an onion.*
4. *Answers will vary.*
5. *Answers will vary.*

Resource Materials

Marmur, J. (1961). *Journal of Molecular Biology, 3,* 208-218.

Teachers Guide to AP Biology Course, AP Development Committee of the College Board, p. 63-65.

Kits

"Spooling Chromosomal DNA." Kit #107. Available from EDVOTEK, Inc., P.O. Box 1232, West Bethesda, MD 20817; 800-EDVOTEK.

"Biotechnology Kit: DNA Extraction." Catalog #21-1100. Available from Carolina Biological Supply Company, 2700 York Rd., Burlington, NC 27215; 800-334-5551.

The Onion Lab:DNA Spooling

STUDENT PAGE 1

Activity Sheet

Introduction

The process of isolating DNA from a cell is the first step for many laboratory procedures in biotechnology. The scientist must be able to separate the DNA from the unwanted substances of the cell gently enough so that the DNA is not broken up or sheared. The procedure you will be doing is a modification of the "Marmur preparation" which is used worldwide in biotechnology laboratories.

Once you have completed the procedure, it is possible to test the material you have isolated with an indicator to determine whether or not it is DNA.

Your teacher has already prepared a "filtrate" for you, made of onion treated with salt, distilled water and dishwashing detergent. We use an onion because it has a low starch content, which allows the DNA to be more clearly seen. The salt shields the negative phosphate ends of DNA which allows these ends to come closer so they can precipitate out of a cold alcohol solution. The detergent causes the cell membrane to break down by emulsifying the lipids and proteins of the cell and disrupting the polar interactions that hold the cell membrane together. The detergent then forms complexes with these lipids and proteins, causing them to precipitate out of solution. You will be altering the filtrate so that you can "spool" DNA out when it precipitates.

DNA Spooling

Materials (obtain from your teacher)

For each lab team

- 1 test tube containing 6 ml of onion filtrate
- 1 plastic pasteur pipette
- 9 ml of ice cold ethanol
- 3.5 ml of meat tenderizer solution
- 1 glass rod (or use your plastic pipette)
- 1 test tube containing 3 ml 4% NaCl solution

Procedure

1. Fill up your pasteur pipet with the meat tenderizer solution until the bulb is half full (3.5 ml). Add the solution to your test tube with the onion filtrate and swirl to mix. *Meat tenderizer contains papain, an enzyme that will clean extra proteins away from DNA.*
2. Immediately add 9 ml of ice cold ethanol to the test tube from step 1 by slowly pouring it down the side of the test tube to create a layer on top. *DNA is not soluble in ice-cold ethanol. When it is added to the mixture, all the components of the mixture except for DNA stay in solution while the DNA precipitates out.*
3. Let the ethanol sit for 2-3 minutes without disturbing it. Bubbles will form and you can watch the DNA precipitate out of the solution.
4. Gently swirl the DNA using a glass rod (or your until the bubbles disappear. You should be able to lift the DNA out of your test tube. (It will look like whitish mucus.)
5. Carefully put the DNA into the test tube containing NaCl solution.

The Onion Lab: DNA Spooling

STUDENT PAGE 2

DNA Testing

Materials (to obtain from your teacher)

Each lab team will need:

- 1 test tube containing 3 ml DNA standard solution
- 1 test tube containing 3 ml distilled water
- 9 ml of diphenylamine solution

Procedure

1. Stir the DNA from the onion with your glass rod to resuspend the DNA into the NaCl solution.
2. Add 3 ml of the diphenylamine solution to the DNA suspension.
3. Add 3 ml of the diphenylamine solution to the test tube containing the DNA standard and 3 ml to the test tube containing distilled water.
4. Place all the tubes in boiling water for 10 minutes and record the color changes. Diphenylamine reacts with deoxyribose compound of DNA to produce a blue color.

Questions

1. Explain the effect that detergent has on the cell membrane.

2. What other vegetables might be used for this lab?

3. Name three properties of DNA that are demonstrated by this lab.

4. Write a short paragraph describing the universality of the DNA molecule.

5. Scientists all over the world use adaptations of this classical method of DNA extraction. The DNA isolated in this manner is routinely used in cloning experiments. This method is commonly used with *E. coli* bacteria and some strains of yeast. What common biological characteristics exist between *E. coli*, yeast and an onion that allow this technique to be effective with all three?

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The Onion Lab: DNA Spooling

STUDENT PAGE 3

Additional DNA Testing Activity (Optional)

If you are familiar with the procedure for electrophoresis you can further test the spooled DNA by resuspending it and running it on an agarose gel.

Materials

Resuspension solution
0.4 ml of 0.25 M EDTA in 100 ml distilled water

Gel loading solution (Blue juice)
0.06 g Bromphenol Blue
10 g sucrose
5 ml SDS in H_2O pH. to 8.5
Bring to total volume of 25.0 ml with sterile distilled water.

Buffer for electrophoresis chamber (1xTBE Buffer)
27 g Tris Base (Sigma T 1503)
13.75 g boric acid
10 µl 0.5 µ EDTA* pH 8.0 (Fisher S-311)
Add distilled water for a total volume of 2.5 l.

Methylene blue stain
.025 g methylene blue (powder) to 100 ml water
Use approximately 100 ml of stain per gel.

Agarose gel
Prepare a 0.4% gel by adding 0.4 g of agarose to 100 ml 1xTBE buffer.

Supplies
10-100 µl micropipet tips
gel box
power supply

Procedure

1. Let the glass rod sit inside a test tube and add 2 ml of the resuspension solution to the tube. Allow the DNA to resuspend for 30 minutes.
2. Add 40 µl of the DNA solution to a 1.5 ml Eppendorf test tube. Mix gently with 5 µl of gel loading solution "blue juice."
3. Run on 0.4% arose gel and stain with methylene blue to visualize bands.
4. Destain gel in water until DNA bands can be visualized.
5. Samples can be run against standard markers to determine the length of sample in base pairs.

* EDTA has a molecular weight of 92.81. A one molar solution (1M) = 92.81 g EDTA plus distilled water for a total volume of 1000 ml. A 0.5 µ solution is 46.41 g EDTA plus distilled water for a total volume of 1,000 ml.

Down and Dirty DNA Extraction

Ability level ..... Grade 7 life science – Grade 12 AP biology
Class periods required ..... 1 1/2 (or adjust to 1)

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Introduction

Students often have trouble understanding the properties of DNA. The extreme length of DNA compared to its thickness makes it appear flexible on the macroscopic level while it is actually stiff and brittle at the microscopic level. In this lab students actually extract and handle DNA, which should reinforce your discussion of DNA structure and function. DNA is extracted from bacterial cells using liquid detergent, and then is precipitated out in alcohol. (Before undertaking this lab students need to understand the importance of sterile technique.)

This inexpensive lab reinforces discussions in the areas of enzymes, cell structure, lipid membranes, supercoiling, denaturation, solubility, viscosity, detergent/lipid interaction, macromolecules (polymers) and the nature of the bacterial genome.

Objectives

At the end of this lab, students should:

1. Understand how to extract a visible mass of DNA.
2. Be familiar with certain physical and chemical properties of DNA, such as solubility, high molecular mass, density, cohesion, etc.
3. Recognize and understand these words: **denaturation, solubility, cohesion.**

Materials

For entire class

- incubator (optional)
- hot water bath (hot plate, large pot or beaker, thermometer)
- Plates with *E. coli* colonies (1 plate for every 2 lab teams)

For each lab team

- 1 eyedropper
- 1 sterile inoculating loop
- 6 ml of ethanol (95%) (keep on ice)
- 2 15 ml culture tubes containing 4 ml of Luria broth*
- 5 ml of 50% solution dish detergent (Palmolive) in water

Teacher Preparation

1. Set up the bacteria cultures. Use sterile technique to plate out *E. coli* before the lab or have your students plate out their own colonies.
2. Prepare a 50% solution of Palmolive dishwashing liquid in water. (Approximately 5 ml per student team).
3. Using sterile technique, the students will add one large scrape of *E. coli* to tubes of Luria broth (or any suitable nutrient broth). Each team of students should inoculate one tube. Incubate these suspensions overnight at 37°C (or for three days with frequent shaking at room temperature). This will provide sufficient numbers of cells for a visible mass of DNA. Immediately before the next class, shake these cultures (to re-suspend the cells).
   (Alternately, you could inoculate a large quantity of Luria broth with an equally large sample of bacteria, incubate as above and dispense these "mature" cultures in individual student aliquots. The students then will begin with student procedure step 2, and only one period would be required to perform the lab.)
4. When the students are ready to extract the DNA, they will need a 65°-75°C hot water bath. The temperature range is critical: 60°C denatures enzymes and 80°C denatures DNA. (For this step, I use a large kitchen pot, a thermometer and a hot plate. The only real requirement is to get a volume of water large enough to prevent the temperature from dropping below 60°C during the denaturing process.)

*Or some suitable nutrient broth

Teaching Tips

1. Review bacterial growth patterns and sterile technique as a Pre-lab activity.
2. Explain to students how detergent disrupts the cell membrane (see student page 1).
3. This procedure does precipitate some proteins (hence the name "Down and Dirty"), but the majority of the precipitate is nucleic acid.

Procedure

(See student pages for detailed protocol.)

1. Students do Background Reading.
2. Day 1: Students incubate *E. coli* cultures.
3. Day 2: Students make a cell suspension and extract DNA.
4. Students answer questions.

Answers to Student Questions

1. *DNA strands can bend a little without breaking, like a stick of spaghetti. DNA strands are so long that this little bit of bending adds up. If you had an uncooked spaghetti stick a mile long, it could bend into a complete circle without breaking. If DNA wasn't so stiff, it wouldn't spool up and would fall off the rod.*

Down and Dirty DNA Extraction

2. *The twisted, double-stranded structure. Imagine the difference between a piece of string and two pieces of string glued together lengthwise and twisted.*
3. *Chemical reactions cause the DNA to bend and fold but retain its stiffness in a new shape. It is like spaghetti that dries in a bent position on the side of the cooking pot, or packaged chinese noodles. Sometimes this phenomenon is referred to as supercoiling.*
4. *If one part of the strand is damaged, the other half can rebuild the damaged part.*

Resource Materials

Kit

DNA Extraction Kit #21-1100. Available from Cabisco Biotechnology, 2700 York Rd., Burlington, NC 27215; 800-334-5551.

Down and Dirty DNA Extraction

STUDENT PAGE 1

Background Reading

Introduction

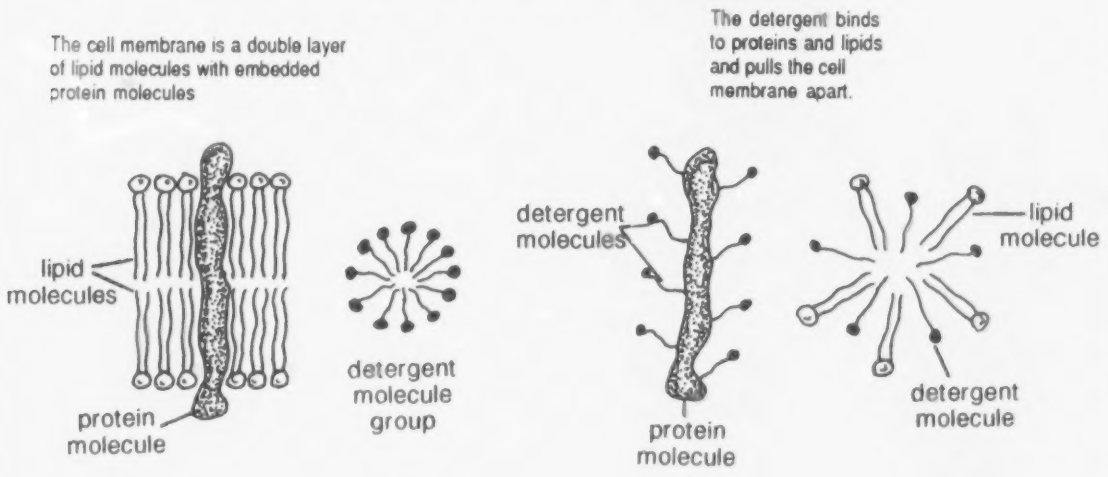
DNA is found in the cells of all living things.

DNA is like an immensely long, uncooked piece of spaghetti; it can bend just so far before it breaks. It looks flexible on the macroscopic scale because it is so long in comparison with its thickness. Even though DNA is strong, it is stiff and brittle and must be handled gently.

How can a cell be opened to extract DNA?

Cell membranes are made of proteins and fats. Just as detergent gently dissolves fats in a frying pan, a little detergent gently dissolves cell membranes:

How Detergent Disrupts the Cell Membrane


The cell membrane is a double layer of lipid molecules with embedded protein molecules
lipid molecules
protein molecule
detergent molecule group
The detergent binds to proteins and lipids and pulls the cell membrane apart.
detergent molecules
protein molecule
lipid molecule
detergent molecule

As the cell membranes dissolve, the cell contents flow out into the liquid where the cells were growing. Since bacterial cells do not have a nucleus, their DNA, the dissolved cell membranes and other contents of the cell become suspended and float in a cell "soup." However, DNA is soluble in water and cannot be seen in this soup. DNA is insoluble in alcohol, however, and will precipitate upward if a layer of alcohol is gently added to the cell soup.

Down and Dirty DNA Extraction

STUDENT PAGE 2

Activity Sheet

Materials (obtain from your teacher)

Each team will need

Day 1

- 1 sterile inoculating loop
- 2 culture tubes containing 4 ml of Luria broth
- 1 wax pencil

Day 2

- 6 ml of 50% detergent solution
- 1 eyedropper
- 6 ml of 95% ethanol
- 1 stirring rod

Procedure

Day 1

1. Obtain individual *E. coli* colonies from a culture plate provided by your teacher. Use a sterile inoculating loop and sterile technique to suspend a 2-3 mm mass of *E. coli* in a 15 ml culture tube containing 4 ml of Luria broth. Label this Tube A.

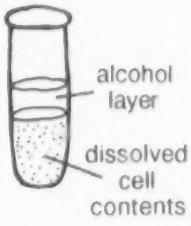
2. Obtain another culture tube containing 4 ml of Luria broth without adding any *E. coli*. Label this Tube B. Incubate both tubes overnight at 37°C or over three days at room temperature with frequent shaking.

Day 2

1. Add to both labeled tubes 3 ml of a 50% solution of dishwashing detergent in water. Shake each tube to assure complete mixing.

2. Your teacher will provide a 65-75°C water bath. Place each tube into the water bath for 15 minutes. **Note:** Maintain water bath temperature accurately. >60°C is needed to destroy enzymes, but temperatures >80°C will denature (break apart) DNA.
3. Use a dropper to carefully pour 3 ml of 95% ethanol on top of the suspension of dissolved cell contents in each tube. (The alcohol should float in a layer on top). Push a glass rod through the alcohol into the suspension, stir and turn. The rod carries a little alcohol into the suspension, and makes DNA come out of solution onto the rod. Keep moving the rod through the alcohol into the cell suspension; each time a little more DNA should appear. Do **not totally mix the two layers** (see diagrams).

Make a layer of ethyl alcohol over the dissolved cell contents.



Move the rod between the layers to begin precipitation.

4. Observe and draw Tubes A and B. Indicate the substances in each tube.

Down and Dirty DNA Extraction

STUDENT PAGE 3

Questions

1. If DNA is a rigid structure, why do the DNA strands appear flexible?

2. What feature of DNA's structure accounts for its stiffness?

3. If DNA is rigid, how does it coil tightly into a small space?

4. What information storage advantage lies in DNA's double helix structure?

Restriction Digest and Analysis of Lambda DNA

Ability level ..... Grade 11 biology, chemistry, Grade 12 AP biology
Class periods required ..... 3 – 4

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Introduction

During this three-day exercise students will work with the bacteriophage lambda, a well-studied virus containing DNA that is approximately 48,514 base pairs long. In the lab, students will digest samples of lambda DNA with three different restriction enzymes: EcoRI, *Bam*HI and *Hind*III. (For a discussion of restriction enzymes, see the following *Sourcebook* activities: "Madam, I'm Adam" and "Restriction Analysis of Pre-Cut DNA"). These enzymes will cut the DNA samples into a number of different size pieces called restriction fragments. One DNA sample will not be cut with restriction enzymes and will serve as a control.

The digested and undigested DNA samples will be loaded into the wells of a 1% agarose gel. The gel will then be placed in an electrophoresis chamber and exposed to an electric current that will flow through the gel. The negatively charged DNA will migrate through the gel toward the positive electrode. The smallest fragments move the fastest and, consequently, will end up closest to the positive pole. The undigested DNA sample should generate only one broad band that is located farthest from the positive electrode.

After the current has run through the gel for a period of time, the gel will be removed and stained with methylene blue, making the DNA bands visible. The bands will be analyzed and compared to a restriction map of lambda DNA which is included with the laboratory materials.

Objectives

At the end of this activity students should be able to:

1. Use a micropipet; operate a microcentrifuge; cast, load and electrophorese an agarose gel; describe how to visualize DNA bands with a staining technique.
2. Compare the lambda DNA bands on a gel to a lambda DNA restriction map.
3. Recognize and understand the following words: **virus, bacteriophage, restriction enzyme, restriction fragments, agarose gel, electrophoresis.**

Materials

For entire class

- microcentrifuge
- 37°C water bath
- cooler with ice (crushed, if possible)
- freezer (frost-free, if possible)
- metric scale (to 1 g)
- Polaroid camera-optional
- Polaroid Type 667 b&w film (optional)
- light table (optional)

For each lab team

- 4 1.5 ml tubes
- 1.5 ml tube rack
- waterproof pen (or substitute labeling system)
- small beaker or plastic foam cup with ice
- 20 µl of 0.5 µg/µl lambda DNA (extra 4 µl included)
- 5 µl 10X restriction buffer (extra 1 µl included)
- 2 µl *Bam*HI restriction enzyme (extra 1 µl included)
- 2 µl *Eco*RI restriction enzyme (extra 1 µl included)
- 2 µl *Hind*III restriction enzyme (extra 1 µl included)
- 1 0.5-10 µl micropipet and tips (approximately 15-20)
- 1 g agarose
- 200 ml 1X TBE buffer
- 125-250 ml flask
- masking tape
- electrophoresis chamber
- power supply (±2 volts)
- 5 µl loading dye (extra 1 µl included)
- gel staining tray
- 25-30 ml of 0.025% (.1g per 399 ml water) methylene blue (or enough to cover the gel in the staining tray)

Teacher Preparation

1. Most of the procedures can be performed by students during the class period. The teacher is responsible for preparing/storing the given aliquots for each lab team.
2. DNA and restriction enzymes are usually shipped in high concentrations for stability. Follow dilution instructions from the supplier. Be careful to maintain these master aliquots from the supply source on ice and use good sterile technique when you prepare student aliquots.
3. It is best to store DNA and enzymes in a non-frost free freezer as they maintain a more stable temperature. If you must use one of the newer types of frost-free freezers, store the enzymes in a plastic foam container. The restriction enzymes are very unstable at high temperatures. Loss of activity will occur if the enzymes are subjected to warm temperatures for any length of time.
4. You will need 1X TBE for the 1% agarose gel solution and to fill the electrophoresis chambers. Use water to calculate the ml of 1X TBE that is needed per gel and for each chamber. Multiply this figure times the number of lab teams you have. Make up and store TBE as a 10X stock solution. Add 1 part of stock solution to 9 parts of distilled water to make the amount of 1X TBE that you calculated in the paragraph above. For example, if you needed 1,000 ml of 1X TBE, add 100 ml of 10X TBE to 900 ml of distilled water.
5. Before the beginning of lab on Day 2, remove the 1.5 ml tubes from the freezer so they will thaw out before class.

Restriction Digest and Analysis of Lambda DNA

Teaching Tips

1. Have students carefully read the procedure before beginning! The quality of their results depends upon good technique and following directions. It is suggested that students practice pipetting, casting gels and hooking up the electrophoresis apparatus. **Keep enzymes on ice.**
2. Photocopy and distribute an extra restriction map to each student. Have them cut out the fragments created by each enzyme and arrange as they will appear on a gel (largest fragment closest to them, smallest fragment the furthest away).
3. Removing the gel tray comb is easier to do after you cover the gel with buffer (prevents tearing of the well).
4. Most gels have at least eight wells, but each lab team needs only four wells. Lab teams could double up on a well to cut down on the number of electrophoresis set-ups, or you could load the extra wells with precut DNA and compare the fragment patterns from the restriction digests with pre-digested DNA.
5. The lambda DNA used in this protocol should be at least 0.50 µg/µl to ensure good methylene blue staining.
6. This protocol was written so teachers could run gels from one day to the next. A 12-volt battery charger will do for a power supply. There are several types of battery chargers available from scientific supply companies. Other approximate voltages and running times:
   - 170 volts – 40 minutes
   - 90 volts – 1-2 hours
   - 50 volts – 2-3 hours (best of the three voltages for lambda DNA)
7. The staining tray can be a plastic foam hamburger box, a weigh boat, or anything large enough to hold the gel yet small enough so that a small amount of stain will cover the gel.
8. Photographing the stained gel is optional; however, it is a big motivating factor for your students as they enjoy comparing their gels with each other. There are Polaroid cameras designed especially for photographing gels, and they work very well. A starting exposure with #667 film should be 1/125 sec at F/16. You may have to increase or decrease your exposure according to the amount of methylene blue still in the gel. Be sure to use a yellow filter to increase contrast as this will make the lighter bands show up much better. You can also photograph the gel with any 35 mm SLR which has a close-up lens attached. Use a color slide film such as *Kodachrome* 64 and the photographs should turn out fine. You can use the slides to show to the whole class.
9. Linear DNA fragments migrate a distance that is inversely proportional to the $\log_{10}$ value of their molecular weight. In this exercise, molecular weight is represented by the number of base pairs. Refer students to the exercise in the lab titled "DNA Restriction Analysis" in *DNA Science* (see **Resources**).

Procedure

(See student pages for detailed protocol.)

1. Students do the Background Reading.
2. Students follow the procedure for **Day 1**, Restriction Digest.
3. Students follow the procedure for **Day 2**, Electrophoresis.
4. Students follow the procedure for **Day 3**, Staining and Analysis.
5. Students answer the Questions.

Answers to Student Questions

1. **Tube A:** *experimental treatment of lambda DNA with the BamHI restriction enzyme; yields four fragments.*
   Tube B: *experimental treatment of lambda DNA with the EcoRI restriction enzyme; yields five fragments.*
   Tube C: *experimental treatment of lambda DNA with the HindIII restriction enzyme; yields six to eight fragments, depending on resolution of the smallest fragments.*
   Tube D: *control treatment; should not have any bands.*
2. *DNA fragments migrated through the gel according to their molecular weight. The smallest fragments travelled the farthest away from the point of origin (the well).*
3. *See table below.*
4. *Students should sketch the diagram of "Staining and Analysis of Gels" from the student pages, then label the bands in descending order from the fragment closest to the origin away from the origin.*
5. *For EcoRI, the 5,643 and 5,804 fragments appear as one band because they are so similar in size; for BamHI, the 6,770 and 6,527 fragments appear as one, as do the 5,626 and 5,505 fragments.*

Answer to Student Question 3.

	*Hind*III	*Eco*RI	*Bam*HI
Origin	XXXX	XXXX	XXXX
	23,130 bp	*21,226*	*16,841*
	9,416	*7,421*	*7,233*
	6,557	*5,643/5,804*	*6,770/6,527*
	4,361	*4,878*	*5,626/5,505*
	2,322	*3,530*	
	2,027		
	**564*		
	**125*		

* small bands that may not resolve

Resources

Micklos, D.A. and Freyer, G.A. (1990). *DNA Science: A First Course in Recombinant DNA Technology*. Cold Spring Harbor Laboratory Press and Carolina Biological Supply Company.

Restriction Digest and Analysis of Lambda DNA

STUDENT PAGE 1

Background Reading

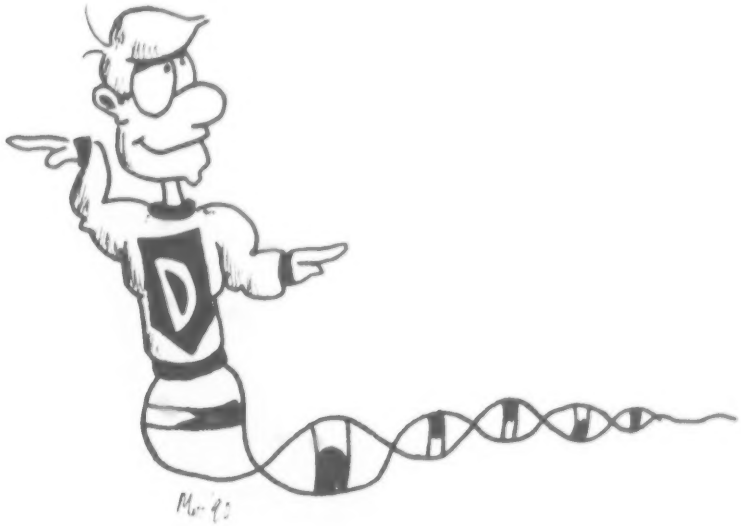
Viruses are generally not considered to be living organisms because they lack basic cell structure and cannot perform independent metabolic functions. Despite these limitations, viruses do exist as parasites of cells. Viruses have the ability to take over the genetic machinery of a cell, resulting in the production of more viruses.

Most viruses only contain between 3 and 240 genes, compared to the roughly 100,000 genes in humans. Since viruses have a relatively simple genetic make-up (or genome), scientists have studied their DNA structure and function and used this information to test theories and develop concepts that apply to the genetics of living organisms. One of the most studied viruses is called bacteriophage lambda.

Bacteriophage lambda is a virus that preys on bacterial cells. Lambda's DNA is approximately 48,514 base pairs in length (the genome of a human cell consists of approximately 3 billion base pairs!). In this activity, you will cut the genome of lambda DNA samples with three restriction enzymes: EcoRI, BamHI and *Hind*III. These enzymes will break the DNA into a number of different sized pieces called restriction fragments.

The restriction fragments will be loaded into the wells of a 1% agarose gel. The gel will then be placed in an electrophoresis chamber and exposed to an electric current that will flow across the gel. Because DNA is a negatively charged molecule, the fragments will move to the positive electrode. The DNA fragments in the gel will migrate through the gel according to their molecular weight. The largest fragments will travel the shortest distance; the smallest fragments will travel the farthest distance away from their point of origin. One of the agarose gel wells will be loaded with a DNA sample that has not been cut with restriction enzymes. This sample will serve as a control.

After the current has run through the gel for a period of time, the gel will be removed and stained with methylene blue, making the DNA fragments visible. The fragment patterns will be analyzed and compared to a restriction map of lambda DNA.



Restriction Digest and Analysis of Lambda DNA

STUDENT PAGE 2

Activity Sheet

Materials

For each lab team

- 4 1.5 ml tubes
- 1.5 ml tube rack
- waterproof pen (or substitute labeling system)
- small beaker or plastic foam cup with ice
- 20 µl of 0.5 µg/µl lambda DNA (extra 4 µl included)
- 5 µl 10X restriction buffer (extra 1 µl included)
- 2 µl *Bam*HI restriction enzyme (extra 1 µl included)
- 2 µl *Eco*RI restriction enzyme (extra 1 µl included)
- 2 µl *Hind*III restriction enzyme (extra 1 µl included)
- 1 0.5-10 µl micropipet and tips (approximately 15-20)
- 1 g agarose
- 200 ml 1X TBE buffer
- 125-250 ml flask
- masking tape
- electrophoresis chamber
- power supply (12 volts)
- 5 µl loading dye (extra 1 µl included)
- gel staining tray
- 25-30 ml of 0.025% (.1g per 399 ml water) methylene blue (or enough to cover the gel in the staining tray)

Procedure

Day 1: Restriction Digest

1. Label four 1.5 ml tubes: *Bam*HI, *Eco*RI, *Hind*III and Control. Place the tubes in the racks for 1.5 ml tubes. **Keep all enzymes and DNA on ice through step 5.** The following chart indicates the amount of reagents to add to each tube. To keep track of each tube's contents, check off each reagent on the chart as you add the reagent to the tube.

Tube	DNA	10X buffer	*Bam*HI	*Eco*RI	*Hind*III	Water
A *Bam*HI	4 µl	1 µl	1 µl	none	none	4 µl
B *Eco*RI	4 µl	1 µl	none	1 µl	none	4 µl
C *Hind*III	4 µl	1 µl	none	none	1 µl	4 µl
D Control	4 µl	1 µl	none	none	none	5 µl

2. Set your micropipet to 4 µl and carefully add 4 µl DNA to each reaction tube. When adding the droplet of DNA to the reaction tube, touch the pipet tip to the side of the tube to deposit a small bead on the inside of the tube.
3. Set the micropipet to 1 µl and carefully add 1 µl of 10X restriction buffer to each tube. Use the same technique as in step 2.
4. Add 1 µl of the appropriate restriction enzyme to the reaction tubes as indicated on the grid. **Use a fresh micropipet tip for each enzyme added.**
5. Add 4 µl of distilled water to the tubes A, B and C; add 5 µl of distilled water to tube D.
6. Close the 1.5 ml tube caps and spin the tubes in the microcentrifuge for about 3 seconds. Caution: Be sure to place the tubes in a balanced configuration.
7. Tap the tubes gently at the bottom in order to mix the reactions. Place the 1.5 ml tubes in a 37° C water bath. Incubate for 20 minutes. **Note:** Since this is the stopping point for today, your teacher may want you to incubate the tubes a little longer before putting them in the freezer. The tubes can incubate for up to 2 hours.
8. Place the 1.5 ml tubes in the freezer until Day 2 of the activity.

Stopping Point for Day #1

Restriction Digest and Analysis of Lambda DNA

Activity Sheet

Procedure

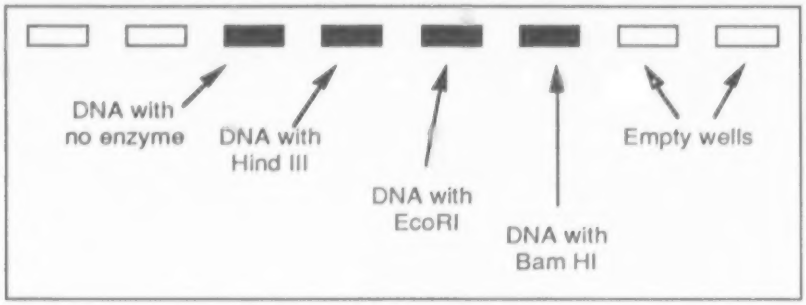
Day 2: Electrophoresis

Preparing the Agarose Gel

1. Weigh 1 gram of agarose powder and place it in a 125-250 ml flask.
2. Add 100 ml of 1X TBE buffer to the flask and stir the mixture.
3. Place the flask containing the agarose mixture on a hot plate or in a microwave. Heat until the solution is completely clear. **Do not cap the flask.** Swirl frequently to prevent charring. Cool solution to 60-70°C before pouring gel.
4. While the mixture cools, tape the ends of the gel tray with masking tape. Place the plastic comb in the slots on the side of the gel tray.
5. Pour the agarose mixture into the gel tray until the comb teeth are immersed about 6 mm (1/4") in the agarose.
6. Allow the agarose to cool about 20 minutes. After it cools completely, carefully remove the comb and tape from the gel tray. Avoid tearing the gel when removing the comb. Each tooth on the comb should leave one neat rectangular well.
7. Place the gel tray in the gel box with the wells at the negative end of the box. The negative end is the black end of the gel box.
8. Add 1X TBE buffer until the wells are barely covered. Cover the gel box and set aside until you are ready to use it.

Preparing for Electrophoresis

1. Add 1 µl of loading dye to each of the 1.5 ml tubes, close the 1.5 ml tube caps and spin the tubes in the microcentrifuge for about 3 seconds. Again, place the tubes in a balanced configuration.
2. Set the micropipet to 10 µl and pipet the entire contents of each tube into each well in the gel (see diagram below).



3. Close the gel box and connect it to the power supply: red to red, black to black. Set the voltage to 12 volts and turn on. Electrophorese for 24 hours.

Stopping Point for Day 2

Restriction Digest and Analysis of Lambda DNA

STUDENT PAGE 4

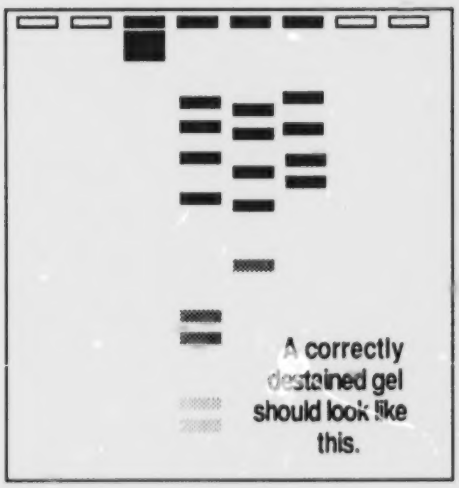
Activity Sheet

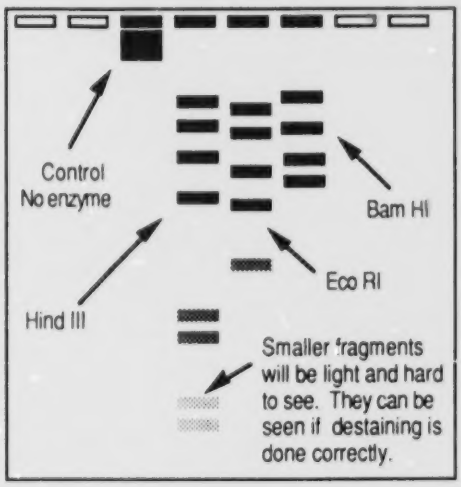
Procedure

Day 3: Staining and Analysis

Staining and Analysis of Gel

1. Unplug the power supply and disconnect the leads. Take the lid off of the gel box.
2. Remove the gel tray and slide the gel into a staining tray.
3. Add enough 0.025% methylene blue to the cover your gel in the gel tray. Let it stand for 20-30 min.
4. Pour the methylene blue out of the gel staining tray back into the stain bottle for re-use later. Add water to the gel staining tray, let it soak for 2-3 minutes, pour off the stained water and replace it with fresh water. Repeat the process 3-5 times. After a couple of rinses, bands begin to appear as darker blue areas. Destaining is critical. If you destain too little, the gel remains dark blue and you cannot distinguish the DNA bands: The bands match the color of their surroundings. If you destain too much, you lose the smallest fragments because these bands are very light.
5. Destain until the bands just become visible, and then destain a couple more times until you see the lightest bands. See the diagram below. Use a light box or overhead projector to see your results more clearly. If you use an overhead projector, do not leave the gel in the bright light for too long: The stain fades rapidly in bright light.




Control
No enzyme
Bam HI
Eco RI
Hind III
Smaller fragments will be light and hard to see. They can be seen if destaining is done correctly.

Restriction Digest and Analysis of Lambda DNA

STUDENT PAGE 5

Questions

1. What was the purpose of

   Tube A:

   Tube B:

   Tube C:

   Tube D:

2. What do the different positions of the bands on the gel represent?

3. Use the enclosed restriction map of lambda DNA to list in the table below the size (in base pairs = bp) of the DNA fragments that make up the bands on your gel.

	*Hind*III	*Eco*RI	*Bam*HI
Origin	XXXX	XXXX	XXXX
	_____bp	_____bp	_____bp
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	
	_____		
	_____		
	_____		

* small bands that may not resolve

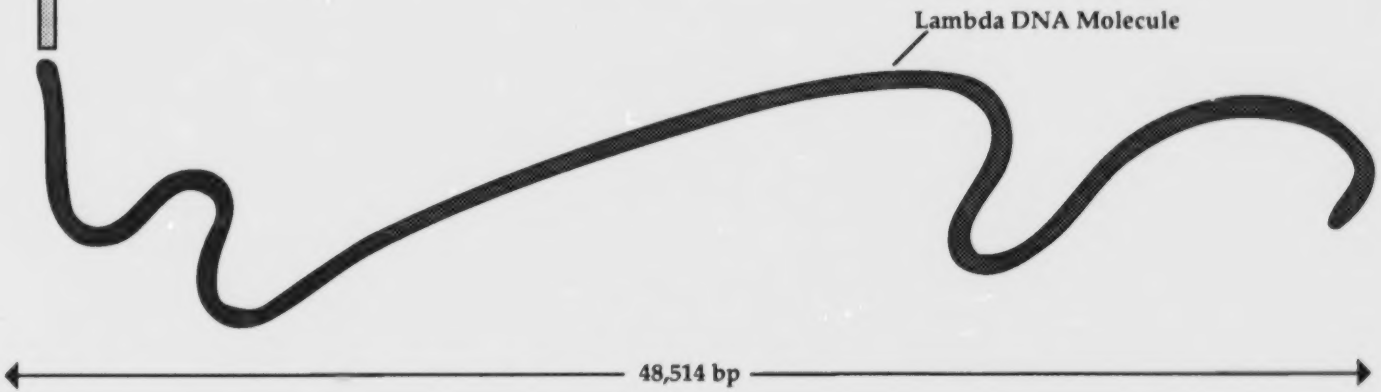
4. On the back of this page sketch the banding pattern of the DNA fragments from your gel. Label origin (well) for the control and each sample treated with an enzyme and the fragment sizes (in base pairs) of each band/lane.

5. Look at the restriction map you used in question #3 above. *Eco*RI and *Bam*HI each have five restriction sites that should yield six fragments, but you only saw five fragments with *Eco*RI and four fragments with *Bam*HI. Why?

Hint: Look at the base pair sizes of the fragments and remember that electrophoresis separates fragments by weight.

Lambda DNA Restriction Map

E. coli bacteriophage Lambda. The Lambda bacteriophage is the best known of the phages. It contains a linear double stranded DNA molecule whose entire 48,514 base pair sequence is known.



Restriction Analysis of Precut Lambda DNA

Ability level .....Grade 9 biology – Grade 12 AP biology
Class periods required .....1 – 2

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Introduction

In this exercise students will take samples of pre-digested DNA from the bacteriophage lambda and run them through a gel for analysis. Each sample has been cut by either the *Hind*III or *Eco*RI enzyme.

Objectives

At the end of this activity students should be able to:

1. Explain the three basic steps in restriction analysis.
2. Analyze DNA fragments of Lambda DNA and compare them to a restriction map.
3. Recognize and understand the following words: **electrophoresis, restriction enzymes (*Hind*III and *Eco*RI).**

Materials

For each lab team

- electrophoresis chamber and gel trays
- power supply (12 to 170 volt)
- digital micropipet ((0.5-10 µl) and tips
- 40-50 ml of melted 1% agarose solution (made with 1X TBE Buffer)
- 4 µl of lambda DNA (0.25 µg/µl) digested by *Eco*RI
- 4 µl of lambda DNA (0.25 µg/µl) digested by *Hind*III
- 2 µl of loading dye
- 25-30 ml of 0.025% methylene blue solution
- masking tape
- destaining trays (large enough to submerge gels)
- glass plate or microscope slide

Teacher Preparation

(This will take at least 2 hours.)

Order sufficient materials for the number of stations (teams) required. It is assumed that other basic materials are already in the classroom. The materials list uses numbers from the Carolina Biological Supply catalog for ease of description, but was developed for the teacher's convenience, and is **not** meant to be inclusive or exclusive of suppliers.

Item	Catalog #
Lambda DNA (EcoRI digest)	21-1474
Lambda DNA (*Hind*III digest)	21-1473
Agarose	21-7080
Loading Dye	21-8200
TBE Buffer	21-9024
Methylene Blue (1%)	21-8290

1. **DNA and loading dye must be refrigerated.** The other reagents and TBE buffer can be stored indefinitely at room temperature.
2. Mix the 172.5 g of TBE (Tris-Borate-EDTA) to 1 liter of distilled water to make a 10X stock solution (ideal for storage). Add 1 unit of 10X buffer to 9 units of distilled water to make a 1X solution. The 1X buffer solution is used in the gel chamber and for dissolving the powdered agarose.
3. Prepare the agarose solution:
   - Determine how much 1% agarose solution will be needed to cast your gels. Each gel requires 30-50 ml of agarose solution, depending on the size of the gel tray. Example: 1 g agarose powder mixed with 100 ml of 1X TBE buffer (from Step #2 above) will yield 100 ml of a 1% agarose solution.
   - Heating the buffer in a clean flask by using a boiling water bath (30-60 minutes) or a microwave (3-6 minutes) will dissolve the agarose powder. **Do not cap** the flask during heating. The gel can be cast when the container is cool enough to hold comfortably. Hardened excess agarose can be remelted for subsequent use. Store the agarose in a closed container at room temperature.
4. Mix 5 ml of 1% methylene blue with 195 ml of distilled water. This will yield 200 ml of 0.025% methylene blue. This amount of methylene is be enough to submerge 6-8 gels (25-30 ml each).

Teaching Tips

1. Depending on the size of the gel chamber, several lab teams could load and run their samples in one gel.
2. Often DC power supplies can be "borrowed" from the physics or chemistry department.
3. An alternative gel box and tray can be made from plastic storage containers and soap dishes as included in Figure 1.
4. Very dexterous students could use graduated microcapillary pipets or other similar alternatives.
5. A common misconception among teachers using the methylene blue staining protocol is to assume that no DNA is present as the gel begins to clear. Continue to destain and the DNA will appear as the background stain diffuses out of the gel. The author suggests that crystal violet be used as a substitute for methylene blue (more rapid background destaining). The protocol and concentration is the same.
6. If necessary, stain for 45-60 minutes, then destain overnight.

Procedure

(See student pages for detailed protocol.)

1. Students do the Background Reading.
2. Students follow the procedure on the Activity Sheet for casting and loading the gel.
3. Students analyze their results by answering the Questions.

Answers to Student Questions

1. *Student fragment patterns should be the same as the ideal pattern. Note that the distance between fragments is a function of running time.*

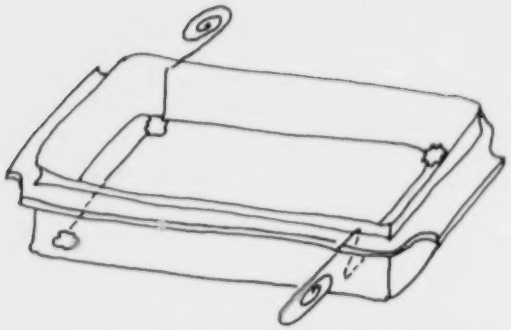
Restriction Analysis of Precut Lambda DNA

2. *No, because the gels only represent the sizes (molecular weights) of the fragments, not where the restriction sites are located. You would need a map of where the sites are located and the size of the fragments (in base pairs). See the map at the end of the lesson Restriction Analysis of Lambda DNA by Larry Tucker.*
3. ***Hypothesis:*** *Restriction enzymes will cut DNA into fragments.*
   Control treatment: *DNA without restriction enzymes*
   Experimental treatment: *DNA with a restriction enzyme*
   Variable: *Restriction enzyme*
   Procedure: *Cut the experimental DNA with restriction enzymes; run the two treatments through the gel electrophoresis technique; stain with methylene blue; the control should not yield any bands, while the experimental treatment should yield a banding pattern.*

Resources

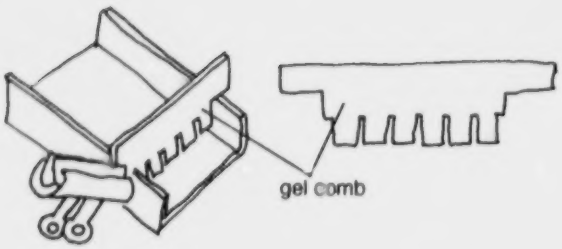
Kits

DNA Restriction Kit 21-1151. Available from Cabisco Biotechnology, 2700 York Rd., Burlington, NC 27215; 800-334-5551.



Inexpensive Electrophoresis Chamber
A plastic sandwich tray with two nichrome wire electrodes held in place with silicon bath caulk. Clip power supply leads to wire loops with alligator clips.

Notes: This box is designed for power supplies of 12-20V The nichrome wire used was purchased from Frey Scientific After a number of runs, the wire will need to be replaced due to the loss of ions to the buffer. The buffer will become clouded but is otherwise unaffected.



Gel Casting Tray and Comb Support.
Tray and comb were cut from a plastic soap dish Comb must *not* contact bottom of casting tray

Figure 1.

Restriction Analysis of Precut Lambda DNA

STUDENT PAGE 1

Background Reading

Over the past two decades, scientists have used restriction analysis to help further their knowledge about the structure and function of chromosomes, genes and DNA. Restriction analysis basically consists of three steps. First, specialized "chemical scalpels" known as restriction enzymes recognize and cut a specific sequence of DNA. After the DNA is cut into fragments, a technique called gel electrophoresis separates the fragments by molecular weight. Finally, the fragments are stained and analyzed.

Restriction Enzymes

Restriction enzymes recognize a specific sequence of nucleotides within the DNA molecule. This sequence is called a recognition site. Once an enzyme finds the correct site, it cuts the sugar-phosphate "backbone" in both strands of the DNA molecule. Sometimes these recognition sites are repeated at several different places along a DNA chain. Using a single enzyme to cut DNA with multiple recognition sites yields several different fragments of equal or unequal lengths.

Gel Electrophoresis

Gel electrophoresis separates DNA fragments by molecular weight. An agar block is cast, then loaded into a chamber that contains a liquid buffer solution. The DNA fragments are loaded into the agar and direct current is passed between wire electrodes at each end of the chamber. The negatively charged DNA fragments are drawn toward the positive pole and repelled by the negative pole. The tangle of agar fibers in the gel allows smaller DNA pieces to migrate farther through the gel than larger DNA fragments. Fragments of the same size stay together in a tight bunch that can be stained and seen as a "band."

Staining

After the DNA fragments are separated, the gel is stained with chemicals that bond to the DNA or to the proteins associated with the DNA. Once the DNA is stained, scientists can analyze the fingerprint-like banding pattern.

Two samples of pre-digested DNA from the bacteriophage lambda are used in this exercise. Each sample has been cut by either the *Hind*III or *Eco*RI enzyme. There are seven *Hind*III restriction sites on lambda DNA, resulting in eight DNA fragments (two of the fragments are small and may appear as one very faint band). The five *Eco*RI restriction sites of lambda DNA yield six fragments of DNA. You will not be able to resolve all of these fragments due to concentration and staining techniques.

Restriction Analysis of Precut Lambda DNA

STUDENT PAGE 2

Activity Sheet

Materials

For each lab team

- electrophoresis chamber and gel trays
- power supply (12 to 170 volt)
- digital micropipet (0.5-10 µl) and tips
- 40-50 ml of melted 1% agarose solution (made with 1X TBE Buffer)
- 4 µl of lambda DNA (0.25 µg/µl) digested by *Eco*RI
- 4 µl of lambda DNA (0.25 µg/µl) digested by *Hind*III
- 2 µl of loading dye
- 25-30 ml of 0.025% methylene blue solution
- masking tape
- destaining trays (large enough to submerge gels)
- glass plate or microscope slide

Procedure

1. Casting the gel:
   - Obtain an electrophoresis chamber and gel tray.
   - Seal the ends of the tray with masking tape. Place the comb at one end of the tray, making sure it **does not** touch the bottom of the tray.
   - Pour enough agarose into the gel tray to cover the lower 1/3 (about 6 mm) of the comb. Allow the agarose to cool.
   - Remove the tape from the gel tray without damaging the ends of the gel. Do not remove the comb at this point. Place the gel tray into the electrophoresis chamber with the "wells" nearest the **negative** electrode end of the chamber.
2. Fill the electrophoresis chamber with 1X TBE buffer. The buffer must completely cover the gel and electrodes.
3. Carefully remove the comb from the gel, leaving the "wells" that you will fill with your DNA samples.
4. Eject two 1 µl samples of loading dye into separate places on a non-absorbent surface, such as a glass plate or microscope slide. Eject one 4 µl sample of DNA (*Hind*III digest) onto one of the drops of loading dye. Mix this DNA and dye by rapidly ejecting and loading the mixture into the micropipet. Load the sample into the appropriate well (avoid puncturing the bottom of the well). Using a fresh pipet tip, repeat this step for the other 4 µl sample of DNA (*Eco*RI digest). Depending on the number of wells in the gel, several student teams may use the same gel. Be sure to sketch the location and identity of the contents of each well.

   Note: It is not necessary to put the tip of the micropipet into the well because the loading dye increases the density of the DNA solution and will help DNA flow into the submerged wells. The loading dye contains two marker pigments. One of the pigments slowly migrates through the gel at a rate similar to a large DNA fragment. The other pigment moves quickly through the gel at a rate similar to a small DNA fragment. When the chamber is charged, the DNA restriction fragments migrate at varying rates between the two marker pigments. For this lab, only the migration of the larger, slower dye sample is relevant. The smaller, faster pigments can even migrate off the gel.
5. Charge the chamber with direct-current electricity. **The electrode nearest the DNA samples in the wells must be connected to the negative pole of the power supply.** Allow the slow marker dye to migrate 2 to 2.5 cm. Depending on the type of power supply, this process will take as long as 24 hrs. (12V), or as little as 40 min. (170V).
6. The final step is to stain the DNA with methylene blue:
   - Flood the gel in a petri dish or other suitable tray-like container with 0.025% methylene blue for 20 minutes.
   - Destain the gel by repeated soakings of tap water. Destaining should continue until the DNA bands become distinct (approximately 30-60 minutes). Continue to destain until the bands become evident. Return unabsorbed stain to the stock bottle.
   - Gels can be photocopied or photographed for a permanent record or refrigerated in sandwich bags for storage. Also, viewing the gels with a yellow filter helps make the bands more visible.

Restriction Analysis of Precut Lambda DNA

STUDENT PAGE 3

Questions

1. Compare your fragment patterns to the ideal fragment patterns for a digest of lambda DNA by *Eco*RI and *Hind*III. Account for differences in separation and band intensity between the experimental gel and the ideal pattern. Draw your pattern and compare it with the ideal digest found on p. 80 of the Teacher's Manual.

Your fragment patterns for *Hind*III and *Eco*RI

*Hind*III

*Eco*RI

2. Can you predict the fragment pattern for a *Hind*III/*Eco*RI digest with only the information (the location of the bands) from the gels you ran? Why or why not?

3. This lab used pre-cut DNA. Theorize how to set a controlled experiment to test if restriction enzymes actually cut DNA. Be sure to describe control and experimental treatments, and define the variable tested.

Liquid Bacterial Transformation

Ability level ..... General level, Grade 9
Biology – Grade 12 AP biology
Class periods required ..... 1 for lab, 2 20-minute student observations 24 & 48 hours after initial lab

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Introduction

In this activity students first demonstrate that *E. coli* are unable to grow in a culture tube containing an antibiotic called ampicillin. Students then insert an antibiotic-resistant gene into *E. coli*. After this transformation, *E. coli* expresses the antibiotic-resistant gene by growing in a medium containing ampicillin. This is phenotypic proof of a genotypic change in the genetic make-up of *E. coli*.

This lab is a economical and reliable protocol that teaches high school students how to change the genetic make-up of bacteria. Low-tech equipment and liquid manipulations are used to decrease cost and increase probability of success.

Objectives

At the end of this lab, students should be able to:

1. Describe a process that changes the genotype of bacteria into an observable phenotype.
2. Identify the control and experimental treatments in this experiment.
3. Recognize and understand the following words: **transformation, plasmid, vectors, genotype, phenotype**

Materials

For entire class

- refrigerator
- autoclave or pressure cooker
- hot water faucet
- ice (crushed or broken up if possible)
- agar culture plates
- *E. coli* (Order a slant culture and streak 1 plate for every two lab teams.)
- 10% Lysol or Clorox sterilizing solution

For each lab team

- inoculating loop
- 5 sterile, empty 15 ml culture tubes, or sterile test tubes (13x100 mm)
- marking pencil
- 3 sterile, calibrated droppers (approximately 4 ml capacity)
- 2 plastic foam coffee cups for hot water and ice baths
- centigrade thermometer
- 15 ml sterile, stock supplies of both Luria broth (LB) and LB+ampicillin (LB+amp)
- 5 ml of 50 mM calcium chloride solution

Teacher Preparation

1. Consult the list below for materials. Order sufficient materials for the number of stations (teams) required. It is assumed that other basic materials are already in the classroom. The list of materials is from Carolina Biological Supply Company (the catalog number is in parentheses), and is not meant to be inclusive/exclusive of other suppliers. *Be sure to double-check catalog numbers before ordering.*
   - 15 ml culture tube (#21-5078) [Alternately, 13x100 mm test tubes can be plugged with cotton and sterilized.]
   - Sterile droppers, calibrated (#73-6895)
   - Calcium chloride (#21-7260)
   - Luria broth (#21-6660J)
   - Luria broth+ampicillin (#21-6661J)
   - Plasmid (pAMP)(#21-1429J)
   - *E. coli* (MM294) (#21-1530J)
2. *E. coli* will be shipped in a slant tube culture that can be refrigerated for 6 months. Prepare 1 streak plate for every 2 lab teams before doing this lab. The bacteria can also be subcultured from the slant tube and frozen for long-term storage.
3. Plasmid DNA is shipped in concentrated, aqueous form (0.2 µg/µl). The plasmid comes with TE buffer, and should be diluted in a ratio of 1 drop of plasmid to 19 drops of buffer. After dilution, the plasmid DNA concentration will be approximately 0.01 µg/µg, which is twice the required DNA concentration for this protocol. Diluted DNA remains viable for long periods if kept under refrigeration.
4. You will need 5 ml of a sterile 50 mM calcium chloride solution per lab team. To prepare this solution, add 2.75 g of calcium chloride to enough distilled water to equal 500 ml. Autoclave this solution at 15 lbs for 15 minutes and refrigerate until ready for use. Keeps indefinitely.
5. Ensure proper treatment of biological wastes. *Any* lab materials that have contacted cells should be placed into a 10% *Lysol* solution (or a 15% *Clorox* solution) overnight. Pressure cooking the materials at 15 lbs for 15 minutes or autoclaving are alternative methods.
6. For transformation, *E. coli* requires only small quantities of plasmid DNA. In developing this protocol, plasmid quantities ranging from one drop to one inoculating loop proved equally successful. To save teacher prep time without sacrificing lab success, it is recommended that teachers dispense one drop of diluted plasmid to each student group.
7. This lab protocol uses calibrated, disposable sterile droppers. They are an economical alternative to micropipets. Described below are specific quantities delivered by these droppers:
   a. One complete dropper. Completely compress the dropper bulb and allow the fluid to enter the dropper and partially fill the bulb (approx. 3.5 ml).
   b. 1.0 ml. Partially depress the bulb and allow the fluid to be drawn into the dropper slightly above the upper mark on the barrel (the 1 ml mark). Expel the excess fluid above the 1 ml mark. Withdraw the pipet from the stock solution. Allow the material to be drawn back into

Liquid Bacterial Transformation

the pipet bulb.

c. 0.5 ml. Similar to "b" above; the 0.5 ml mark is located midway up the barrel.

d. One drop. One drop equals approximately 40 µl.

e. The order of operations which use these droppers is critical. Steps must not be completed out of order.

Teaching Tips

1. Students should study the protocol before beginning. Success depends on timely and accurate movement through the steps.
2. Expected results:
   - Tubes A+, B and D: Cell growth should occur – indicated by *cloudiness*. Tubes B and D will cloud up within 24 hours; Tube A+ will cloud within 48 hours.
   - Tubes C– and E: Cell growth should NOT occur – indicated by *clear fluid*.
3. The time difference between tubes B and D vs. tube A+ is due to the nature of ampicillin. Ampicillin prevents the formation of cell walls. In tubes B and D, cells grew at a relatively unrestricted rate because ampicillin was absent. In tube A+, the bacteria reproduced only *after* the resistance gene began to manufacture protein to offset ampicillin. In other words, transformants cloud after a brief dormant period.

Procedure

(See student pages for detailed protocol.)

1. Students read Introduction, study protocol and assemble their materials.
2. Students follow procedure for inoculating tubes with bacteria, adding plasmid, subjecting bacteria to heat shock and adding ampicillin.
3. Students answer Questions.

Answers to Student Questions

Table (student pg. 2)

Tube	*E. coli*	pAMP	LB	LB+AMP	Expected Growth?	Observed Growth(hrs) 24	48
A+	yes	yes	no	4 ml	*Yes*	*No*	*Yes*
B	yes (1/2 A+)	yes (1/2 A+)	4 ml	no	*Yes*	*Yes*	*Yes*
C–	yes	no	no	4 ml	*No*	*No*	*No*
D	yes (1/2 C-)	no	4 ml	no	*Yes*	*Yes*	*Yes*
E	no	no	4 ml	no	*No*	*No*	*No*

Questions

1. Control tube (letter): *Tube E*
   Experimental tube (letter): *Tube D*
   Accept hypothesis. If reject, students should hypothesize why. Answers will vary.

2. Control tube (letter): *Tube D*
   Experimental tube (letter): *Tube C–*
   Accept hypothesis. If reject, students should hypothesize why. Answers will vary.

3. Control tube (letter): *Tube B*
   Experimental tube (letter): *Tube A+*
   Accept hypothesis. If reject, students should hypothesize why. Answers will vary.

4. Control tube (letter): *Tube C–*
   Experimental tube (letter): *Tube A+*
   Accept hypothesis. If reject, students should hypothesize why. Answers will vary.

5. *Answers will vary.*

Liquid Bacterial Transformation

STUDENT PAGE 1

Activity Sheet

Introduction

Bacterial transformation is the uptake of foreign DNA by bacterial cells. This phenomenon occurs infrequently in nature, perhaps due to its inefficiency. However, in 1970 M. Mandel and A. Higa discovered a process for increasing the ability of *E. coli* cells to transform. Rapidly growing cells were suspended in cold calcium chloride and saturated with plasmid DNA. After exposing them to brief heat shock (40-42°C), the cells transformed more efficiently. This lab exercise is based on that procedure.

In this lab, a small self-replicating circle of extrachromosomal DNA, which is called a plasmid, represents foreign DNA. The plasmid we will use is called pAMP because it contains a gene for ampicillin resistance. Plasmids are called vectors because they transport genetic material into and out of cells. Plasmid vectors used in research are frequently engineered to contain DNA for both a desired trait (such as the manufacture of enzymes or hormones) and an identifying trait for selecting the organisms that have taken up the desired gene. Since our goal is to prove only that transformation occurred, our vector (pAMP) contains only the selection gene.

Materials

- inoculating loop
- 5 sterile, empty 15 ml culture tubes, or sterile test tubes (13X100 mm)
- marking pencil
- 3 sterile, calibrated droppers (approximately 4 ml capacity)
- 2 plastic foam coffee cups, one for hot water and one for ice
- centigrade thermometer
- 15 ml stock supplies of both Luria broth (LB) and Luria broth+ampicillin (LB+amp) in separate, sterile tubes
- 5 ml of 50 mM calcium chloride solution

Procedure

1. Label one sterile 15 ml tube as A+ (with plasmid) and another C– (without plasmid). Using sterile technique and a sterile dropper, add 0.5 ml of calcium chloride solution to each labeled tube. Place both tubes in an ice-filled cup.

2. Inoculate the A+ and C– tubes with bacteria:
   a. Locate a bacterial colony (4-5 mm in diameter) from a plate.
   b. Flame the inoculating loop until red hot, cool it by touching the agar of the plate *away* from the colonies, and gently scrape the colony of cells into the loop. Be careful not to touch the bacteria with the hot loop, and avoid pulling up agar with your cell mass. Agar interferes with transformation. If you cannot find an individual colony, scrape enough cells to fill the loop.
   c. Stir this loopful of bacteria into tube A+ and repeat steps "a" and "b" for tube C–. It is important to *completely* suspend your cells in the calcium chloride solution. Do not worry about physically damaging the cells. Be aggressive. Always reflame the loop after completing a step.
   d. Place both tubes in the ice cup.

3. Take tube A+ to your teacher and receive one drop of plasmid DNA (pAMP). Gently swirl to mix your cell suspension and the DNA drop. Return the tube A+ to the ice cup, and incubate both tubes on ice for 20 minutes.

(continued on next page)

Liquid Bacterial Transformation

STUDENT PAGE 2

4. While tubes A+ and C– are on ice, label 3 additional tubes B, D and E. Examine the table below. Fill in your predictions in the "Expected Growth" column.

Tube	*E. coli*	pAMP	LB	LB+AMP	Expected Growth?	Observed Growth(hrs) 24	48
A+	yes	yes	no	4 ml			
B	yes (1/2 A+)	yes (1/2 A+)	4 ml	no			
C–	yes	no	no	4 ml			
D	yes (1/2 C-)	no	4 ml	no			
E	no	no	4 ml	no			

5. After tubes A+ and C– have been in the ice bath for 20 minutes, place each tube in a 42°C water bath for 90 seconds. Use your thermometer to adjust the hot/cold water tap faucet to 42°C. **Fill your cup with 42°C water and place tubes A+ and C- in the hot water bath for exactly 90 seconds. Note:** To avoid temperature drop in your hot water bath, try to time this step to coincide with the end of the incubation period of step 3. **Do not let your water temperature drop below 42°C.** This "heat shock" step helps establish the plasmids in the bacterial cells.

6. Immediately after the 90-second heat shock, return tubes A+ and C– to the ice for one minute. This allows the bacterial cells to recover from the shock of the temperature change.

7. Use a second sterile dropper to quickly transfer half the contents of tube C– (without plasmid) into tube D. (Be sure you are using sterile technique throughout this step.) Then, transfer half the contents of tube A+ (with plasmid) into tube B. **You must transfer the materials of tube C– first before touching the dropper to A+ cells to avoid contamination. Discard the dropper properly.**

8. Use a *third* sterile dropper to add one full dropper (approx. 3.5 ml) of Luria broth **without ampicillin** to tubes E, then D, then to B. Use the same dropper to add one full dropper of Luria broth **plus ampicillin** to tube A+ and tube C–. **You must transfer the Luria broth without ampicillin first to avoid contamination.**

9. Place tubes A+, B, C–, D and E in a 37°C incubator. Observe and record the results for each tube at 24 and 48 hours in the table provided (see step #4). If a tube becomes cloudy and has a smell, *E.coli* have grown in the tube. If transformation occurred in tube A+, it will cloud more slowly than the other tubes. Use the test tube diagrams in Figure 1 to illustrate your results after 48 hours.

Liquid Bacterial Transformation

STUDENT PAGE 3

Questions

One of your objectives in this lab was to create a genotypic change in *E. coli* that could be seen as a phenotypic change. Ampicillin is an antibiotic that destroys bacteria. A gene for resistance to ampicillin was added to the normal complement of bacterial DNA, which changed (transformed) the genotype. The altered bacteria were then exposed to an environment to see if they would express this change as a phenotype.

This activity was to test several hypotheses with control and experimental treatments. For each of the hypotheses below, look at the table on student page 2 and write in answers to the following questions:

1. **Hypothesis #1: Untransformed bacteria grow in Luria broth (nutrient).**

Control tube: _____

Experimental tube: _____

Accept or reject hypothesis? If reject, why?

2. **Hypothesis #2: Untransformed bacteria do not grow in ampicillin.**

Control tube: _____

Experimental tube: _____

Accept or reject hypothesis? If reject, why?

3. **Hypothesis #3: Transformed bacteria grow in both Luria broth and LB plus ampicillin.**

Control tube: _____

Experimental tube: _____

Accept or reject hypothesis? If reject, why?

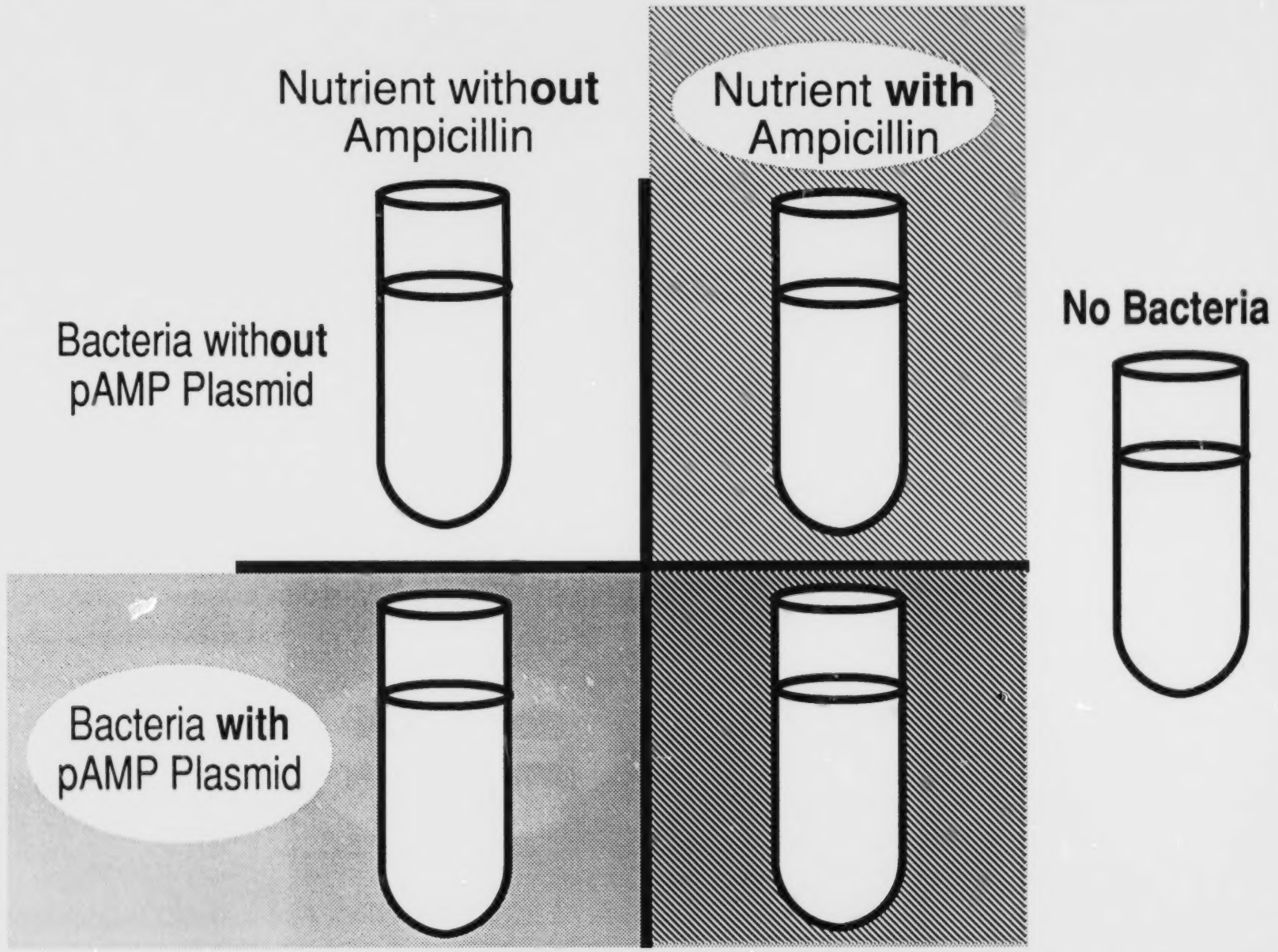
4. **Hypothesis #4: Untransformed bacteria do not grow in LB plus ampicillin; transformed bacteria do grow in LB plus ampicillin.**

Control tube: _____

Experimental tube: _____

Accept or reject hypothesis? If reject, why?

5. Using the results of this lab, discuss the relationship of genotype to phenotype.


Nutrient without Ampicillin
Nutrient with Ampicillin
Bacteria without pAMP Plasmid
No Bacteria
Bacteria with pAMP Plasmid

110

111

Plated Colony Transformation

Ability level ..... Grade 9 biology – Grade 12 AP biology
Class periods required ..... 1 for the lab, 1/2 24 hours later for observation

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Introduction

The naturally occurring phenomenon of bacterial transformation has been of critical importance in the development of molecular biology. Today it is one of the most widely used and versatile tools in genetic engineering.

Transformation is common in some bacteria, but other bacteria, including *E.coli*, do not take up naked DNA readily. In 1970, it was discovered that certain conditions can help bacterial cells absorb exogenous DNA. These conditions are:

- Cells are rapidly growing.
- Cells are suspended in cold calcium chloride.
- Heat-shocking the cold suspension of cells to 40-42° C.

In addition, intact plasmids are much more readily absorbed than DNA fragments.

In this lab activity, students will conduct a controlled bacterial transformation experiment. Students will attempt to cause a wild type strain of *E. coli* to incorporate *and* express a plasmid containing an ampicillin-resistance gene (pAMP). Ampicillin is an antibiotic that prevents *E. coli* from constructing cell walls. When the wild type strain genetically changes into an ampicillin-resistant strain, the bacteria are said to be *transformed*. If transformation occurs, the students will observe colonies of transformed bacteria growing on agar plates containing ampicillin.

Adapted with permission from *DNA Science: A First Course in Recombinant DNA Technology* by David A. Micklos, DNA Learning Center, Cold Spring Harbor Laboratory, and Greg A. Freyer, Columbia University College of Physicians and Surgeons, ©1990, Cold Spring Harbor Laboratory Press and Carolina Biological Supply Company. Based on a protocol published by Douglas Hanahan, University of California, San Francisco, California.

Objectives

At the end of this activity, students should be able to:

1. Explain how a genotypic change can affect the phenotype of an organism.
2. Define cell membrane permeability and discuss how it can be manipulated in bacterial transformation.
3. Explain how they would set up a controlled experiment to test some of the limiting factors affecting bacterial growth or transformation, such as temperature, nutrient availability, calcium chloride and heat shocking.
4. Describe some sterile techniques that are used to handle bacteria.
5. Recognize and understand the following words: **transformation, membrane permeability, ampicillin, plasmids.**

Materials

For entire class

- *E.coli* (MM294); one streak plate for every two lab teams
- 1.5 ml tube rack; one for every 3-4 lab teams
- incubator (37°C)
- autoclave (or pressure cooker)
- flame source (Bunsen burner, alcohol lamp, etc.)
- ice (crushed, if possible)
- 100 µl of sterile water or TE buffer
- 10% Clorox or Lysol in spray bottles (2-3)
- microbial and non-microbial waste containers

For each lab team

- waterproof marker or marker and tape
- 2 1.5 ml microcentrifuge tubes
- 100-1,000 µl pipet and tips (or sterile transfer pipet)
- 0.5-10 µl pipet tips
- 500 µl 100 mM calcium chloride
- inoculating loop
- 2 plastic foam cups
- 0.25 µg pAMP
- 2 LB plates
- 2 LB + Amp plates
- 500 µl Luria broth
- bacterial spreader (or bent glass stirring rods)
- alcohol in an agar plate (enough to wet the end of the spreader)

Teacher Preparation

(requires at least 1 hour)

1. Order a slant culture of *E. coli*. Several days before class, streak the *E.coli* cells on several LB plates and incubate for about 24 hours at 37°C. For a student activity, students can scrape single colonies from the starter plate to make their own plates.
2. This transformation protocol requires 0.25 µg pAMP plasmid. The pAMP plasmid is available from several different suppliers with concentrations ranging from 0.2 to 0.6 µg/µl, which is too concentrated. For example, if you added 5 µl (from "Student procedure," step 9) of 0.5 µg/µl pAMP to tube "+", the amount of pAMP would be 2.5 µg instead of 0.25 µg, or a factor of 10X too much DNA. To dilute this particular concentration of pAMP, add 5 µl of pAMP to 45 µl of TE buffer or sterile water. This will yield (10) 5 µl aliquots that can be distributed to students.
3. You will need 500 µl (0.5 ml) of a sterile 100 mM calcium chloride solution per lab team. 11 g of calcium chloride in 1,000 ml of distilled water is a 100 mM solution. Since you will probably only need about 50 ml of calcium chlo-

Plated Colony Transformation

ride per class, add 1.1 g of calcium chloride to 100 ml of distilled water. The excess 50 ml of calcium chloride will keep for two months at room temperature if maintained in sterile condition.

4. Distribute to students photocopies of the overhead master at the end of this lesson.

Teaching Tips

1. To save plates, have each lab group do only one LA control: either "LA+" or "LA–" (see "Student procedure, step #11).
2. Before doing the lab, students should review and understand:
   - the scientific method.
   - basic sterile techniques.
   - protein synthesis and the relationship of proteins to observable traits.
   - basic structure and function of prokaryotic cells.

Procedure

(See student pages for detailed protocol.)

1. Students read Background Reading.
2. Students follow transformation procedure.
3. Students answer Questions.

Answers to Student Questions

1. *E. coli* **not** exposed to pAMP	*shaded*	*not shaded*
Wild type *E. coli* **exposed** to pAMP	*shaded*	*shaded*

2. *E. coli* **not exposed** to pAMP on a plate **without** ampicillin:
   bacterial nutrient

   E. coli **not exposed** to pAMP on a plate **with** ampicillin:
   The bacteria should not grow on bacterial nutrient that contains the antibiotic ampicillin.

   E. coli **exposed** to pAMP on a plate **without** ampicillin:
   Not all of the bacteria will be transformed with the ampicillin-resistant gene; however, since there is no ampicillin in the nutrient, both the transformed and untransformed bacteria can grow.

   E. coli **exposed** to pAMP on a plate **with** ampicillin:
   Bacteria that are transformed with pAMP will grow on a plate containing ampicillin. Even some of the bacteria that are not transformed in this lab will grow because the transformed bacteria secretes the enzyme that destroys ampicillin, therefore allowing non-transformed bacteria to be "shielded."

Note: If students do not obtain the desired results, have the students retrace their steps and discuss with the class.

Resources

Kit

Colony Transformation Kit #21-1142. Carolina Biological Supply Company, 1-800-334-5551.

Printed materials

Micklos, D.A. & Freyer, G.A. (1990). *DNA science: A first course in recombinant DNA technology.* Cold Spring Harbor Laboratory Press and Carolina Biological Supply Company. 477 pp.

Plated Colony Transformation

STUDENT PAGE 1

Background Reading

Transformation means "change." In molecular biology, transformation refers to a form of genetic change in which bacteria take up free DNA from the environment and incorporate it into their own genetic material. Most bacteria do not "transform" readily, but researchers have discovered ways to enhance the uptake of DNA so that this natural means of genetic engineering will occur more readily in laboratories.

You will be transforming a bacterium, *E. coli* , that lives in the human gut. Genetically, *E. coli* is relatively simple and well understood. Its genetic material consists mostly of one large circle of DNA between 3-5 million base pairs in length, and some small loops of DNA called plasmids usually ranging from 5,000-10,000 base pairs, but sometimes even larger. Plasmids are important to genetic engineers because they can be used as "gene taxis" in transformation events.

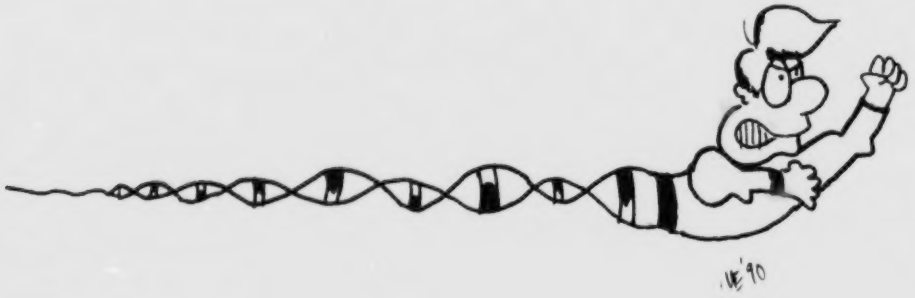
Like most bacteria, *E. coli* do not take up DNA readily. Certain conditions listed below help bacterial cells absorb plasmids. The conditions are:

- Cells are rapidly growing (in the mid-log growth phase).
- Cells are suspended in cold calcium chloride.
- After foreign plasmid DNA is added to rapidly growing cells in cold calcium chloride, the suspension of cells is briefly heated to 40-42°C.

No one knows exactly how or why these conditions make the bacteria take up free DNA. It is reasonable to assume that rapidly growing cells would require cell membranes that are relatively permeable to nutrients (or plasmids) coming into the cell and waste products being excreted. Exactly how the other two conditions listed above might change membrane permeability remains somewhat of a mystery.

In this lab you will cause a wild type strain of *E. coli* to incorporate and express a plasmid containing an ampicillin resistance gene. The plasmid is called pAMP ("p" for plasmid, "AMP" for ampicillin). If transformation occurs, you will observe colonies of transformed bacteria growing on agar plates containing ampicillin.

Ampicillin is an antibiotic that prevents *E. coli* from constructing cell walls. Ampicillin is non-functional as a building block, so the new bacterium builds flawed cell walls. The cell walls fall apart, and the new bacterium does not survive. If a strain of *E. coli* has an ampicillin-resistance gene, the strain survives because the gene directs the production of an enzyme to block the action of ampicillin. When the wild type strain genetically changes into an ampicillin-resistant strain, the bacteria are said to be *transformed*.



Plated Colony Transformation

STUDENT PAGE 2

Activity Sheet

Materials

For entire class

- *E. coli* (MM294); one streak plate for every two lab teams
- 1.5 ml tube rack; one for every 3-4 lab teams
- incubator (37°C)
- autoclave (or pressure cooker)
- flame source (Bunsen burner, alcohol lamp, etc.)
- ice (crushed, if possible)
- 100 µl of sterile water or TE buffer
- 10% Clorox or Lysol in spray bottles (2-3)
- microbial and non-microbial waste containers

For each lab team

- waterproof marker or marker and tape
- 2 1.5 ml microcentrifuge tubes
- 100-1,000 µl pipet and tips (or sterile transfer pipet)
- 0.5-10 µl pipet tips
- 500 µl 100 mM calcium chloride
- inoculating loop
- 2 plastic foam cups
- 0.25 µg pAMP
- 2 LB plates
- 2 LB + Amp plates
- 500 µl Luria broth
- bacterial spreader (or bent glass stirring rods)
- alcohol in an agar plate (enough to wet the end of the spreader)

Procedure

1. Mark one sterile 1.5 ml tube "+"; mark another "–". Plasmid DNA (pAMP) will be added to the "+" tube; none will be added to "–" tube.
2. Using a 100-1,000 µl micropipet and sterile tip (or sterile transfer pipet), add 250 µl calcium chloride solution (0.1 M) to each tube ("+" and "–").
3. Place both tubes on ice.
4. Use the following procedure to transfer one or two large (3 mm) colonies from an agar plate to the "+" tube:
   - Sterilize an inoculating loop in a Bunsen flame until it glows red hot. Continue to pass the lower 1/3 of the shaft through the flame.
   - Stab the loop several times into the agar to cool the loop. **Do not touch the bacterial colonies until you have cooled the loop.**
   - Scrape into the loop one or two 3 mm bacterial colonies, but be careful not to transfer any agar (impurities in the agar can inhibit transformation).
   - Immerse the loop tip into the calcium chloride solution in tube "+", and **VIGOROUSLY** tap the loop against the tube's wall to dislodge the cell mass. Hold the tube up to the light to observe if the cell mass fell off the loop.
   - Reflame the loop before setting it down.

Note: It is important to use a sufficiently large cell mass in step #4. If the plates do not contain enough 3 mm colonies, scrape up several smaller colonies. Plates incubated 24 hrs at 37°C or 48 hrs at 25°C, should contain sufficiently large colonies.

5. Immediately resuspend the cells in the "+" tube by repeatedly pipeting in and out with the 100-1,000 µl micropipet and sterile tip (or sterile transfer pipet). Hold the tube up to the light, and carefully inspect it to see that the suspension is homogeneous. **NO VISIBLE CLUMPS OF CELLS SHOULD REMAIN.**

Keep your nose and mouth away from the tip end when pipeting the suspension culture to avoid inhaling any aerosol that might be created.

Note: Inspect your tubes for complete resuspension of cells. This step is critical to obtaining good results. Resuspending cells first in the "+" tube allows the cells to preincubate for several minutes at 0°C. while you transfer and suspend cells to the "–" tube.

6. Return the "+" tube to ice, along with the "–" tube.
7. Transfer and suspend a second mass of cells to the "–" tube, as described in Steps 4-5 above.
8. Return the "–" tube and "+" tube to ice. Incubate the cells on ice for 10-15 min.
9. Use 1-10 µl micropipet to add 5 µl pAMP to the "+" tube. Tap the tube with your finger to mix. Avoid making bubbles in the suspension, or splashing the suspension up the sides of tube.
10. Return the "+" tube to ice, along with the "–" tube. Incubate both tubes on ice for an additional 15 min.

Plated Colony Transformation

STUDENT PAGE 3

11. While the cells are incubating, label two LA and two LA/AMP plates with lab group name(s) and the date. Make sure the plates are at room temperature.
    - Label 1 plate "LA+" and 1 plate "LA/AMP+"
    - Label 1 plate "LA–" and 1 plate "LA/AMP–"

Note: LA plates are plain agar plates; LA/AMP are plates that contain agar and the antibiotic ampicillin.

12. Following incubation, heat shock the cells. It is essential that the cells receive a sharp and distinct shock:
    - Carry the ice beaker containing the tubes to the water bath.
    - Remove both tubes from ice; immerse them in a 42°C water bath for 90 sec.
    - After heat shock, immediately return the tubes to ice.
13. Let the tubes stand on ice for at least one additional minute.
14. Set the tubes in a test tube rack at room temperature.
15. Use a 100-1,000 µl micropipet and sterile tip (or transfer pipet) to add 250 µl Luria broth to each tube. Gently tap tubes to mix. Incubate at 37°C. for 30 min. *or* at room temperature overnight.
16. Set up the matrix below to use as a checklist as you spread the "+" and "–" cells on each type of plate:

	LA	LA/AMP
Control cells (–)	100 µl	100 µl
Transformed cells (+)	100 µl	100 µl

17. Use a 100-1,000 µl micropipet and sterile tip (or transfer pipet) to add 100 µl of cell suspension from the "–" tube to the "LA–" plate, and another 100 µl to the "LA/AMP–" plate. Do not let the suspension sit on the plate too long before spreading; if too much liquid is absorbed by the agar, the cells cannot be evenly distributed.

    Keep your nose and mouth away from the tip end when pipeting the suspension culture to avoid inhaling any aerosol that might be created.

18. Plate the cells with a sterile spreader. The object is to evenly spread out and isolate the cells on the agar surface so that each cell gives rise to a distinct colony:
    - Dip the spreader in ethanol, then pass it through a Bunsen flame only long enough to ignite the alcohol. Remove the spreader from the flame (spreading rod will become too hot if left in the Bunsen flame).
    - Allow the alcohol to burn off.
    - Lift the lid from " LA–" plate, but do not set the lid down on the lab bench.
    - Cool the spreader by touching the agar away from the 100 µl cell suspension that was pipetted on to the plate during step #17. It is essential to cool the spreader before touching it to cells.
    - Touch the spreader to the cell suspension, and gently drag it back and forth several times across the surface of agar.
    - Rotate the plate a 1/4 turn, and repeat the spreading motion.
    - Replace the plate lid.
19. Repeat Step 18 to spread the cell suspension on the "LA/AMP–" plate.
20. Use a new sterile tip (or transfer pipet) to add 100 µl of cell suspension from the "+" tube to the "LA+" plate, and another 100 µl to the "LA/AMP+" plate.
21. Repeat Step 18 to spread the cell suspensions on the "LA+" and "LA/AMP+" plates.
22. **REFLAME THE SPREADER ONE LAST TIME.**
23. Place the four plates upside down in a 37°C incubator. Incubate for 12-24 hrs. If an incubator is not available, incubate plates at room temperature for two days.
24. After initial incubation, store the plates at 4°C to arrest *E. coli* growth and slow growth of any contaminating microbes or fungi.
25. **TAKE TIME FOR RESPONSIBLE CLEANUP. Segregate microbial and non-microbial waste products for proper disposal. Bacterial cultures, *as well as* tubes, pipets and micropipet tips that have come into contact with the cultures, are microbial waste. Autoclave or treat microbial waste with antimicrobial solutions (10%), such as Clorox or Lysol. Non-microbial waste can be disposed of in a normal fashion.**

Plated Colony Transformation

STUDENT PAGE 4

Questions

1. Draw a circle representing an agar plate in each of the four squares in the grid below. Shade the circles to indicate the plates you predict will show bacterial growth after incubating the plates at 37°C for 24 hours:

	Plate **without** Amp	Plate **with** Amp
E. coli **not** exposed to pAMP		
Wild type *E. coli* **exposed** to pAMP		

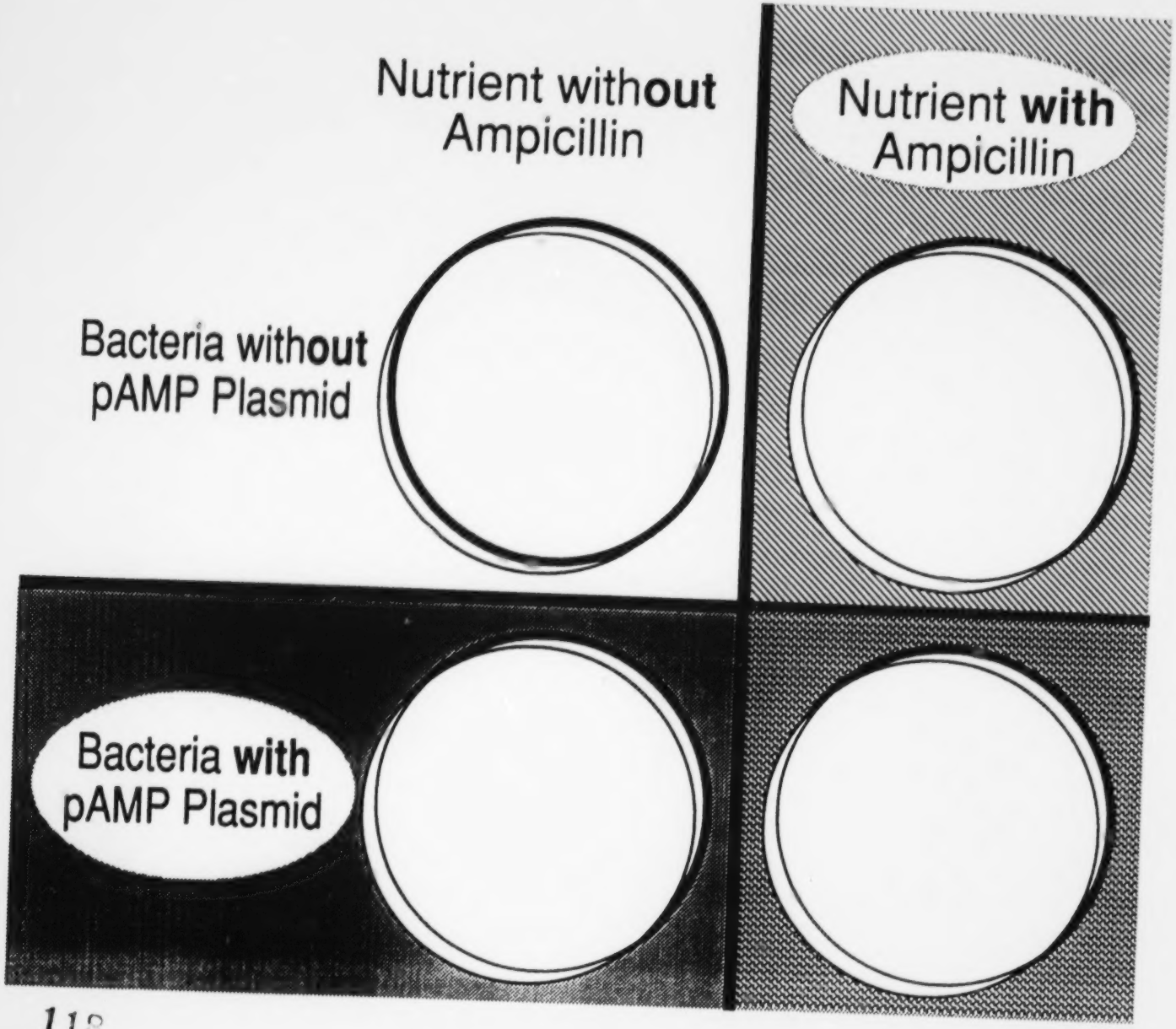
2. Using the diagram on the next page, shade the plates from your lab team that show bacterial growth. Compare your predicted results in question #1 above with the actual results you observed. Explain why growth or non-growth occurred for each plate.

   E. coli **not exposed** to pAMP on a plate **without** ampicillin:

   E. coli **not exposed** to pAMP on a plate **with** ampicillin:

   E. coli **exposed** to pAMP on a plate **without** ampicillin:

   E. coli **exposed** to pAMP on a plate **with** ampicillin:


Nutrient without Ampicillin
Nutrient with Ampicillin
Bacteria without pAMP Plasmid
Bacteria with pAMP Plasmid

Viruses as Vectors

Ability level ..... General biology
Class periods required ..... 1 class discussion/2 homework assignments

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Introduction

In this lesson students read and work through a series of questions and worksheets. These exercises cover virus structure and function, lytic and lysogenic life cycles, general and restricted transduction. Suggestions for supplementary readings are included.

Objectives

At the end of this activity, students should be able to:

1. Demonstrate an understanding of how virus structure complements its function as a obligate parasite.
2. Compare and contrast lytic and lysogenic life cycles.
3. Explain the process of transduction.
4. Distinguish between general and restricted transduction.
5. Describe how transduction occurs in nature as well as how it can be a useful tool in genetic engineering.
6. Recognize and understand these words: **bacteriophage, obligate parasite, lytic cycle, restricted transduction, lysogenic cycle, retrovirus.**

Materials

For each student

- red, green, black colored pencils
- copies of Background Readings and Worksheets

Teacher Preparation

Make copies of Background Reading and Worksheets.

Teaching Tips

Supplementary readings and/or textbook material can also be used to help students with the worksheets.

Procedure

Part 1: Virus Structure, Function and Life Cycle

Students do Background Reading.
Students do Worksheet: label diagrams and answer questions.

Part 2: Virus Transduction

Students do Background Reading.
Students do Worksheet: label diagrams and answer questions.

Answers to Student Questions

Part 1.

1. *In the lytic cycle, the virus (actiing as a virulent phage) enters the bacterial cell, takes over the host's metabolic machinery and compels it to make new viral nucleic acid and proteins. The virus structures are assembled and then new viruses lyse the host cell and escape to infect other cells.*
   In the lysogenic cycle the virus (acting as a non-virulent phage) enters the bacterial cell and becomes integrated into the host chromosome. Every time the host replicates, it replicates the bacterial nucleic acid as well as its own. In this way many cells have the virus nucleic acid.
2. i. *A*
   ii. *C*
   iii. *B*
3. *Answers will vary depending on student background. The host cell will accept viruses (they have evolved receptors that "match" sites on the host for attachment). The molecular biology of viruses has been studied extensively. The viral genome contains strong promotors; it is possible to ensure efficient expression of the foreign genes inserted into viral DNA.*
4. *See diagrams provided with the reading.*

Part 2.

1. *The virus acts as a vector (vehicle) in carrying nucleic acid from one source to a new host.*
2. *In general transduction the bacterial host chromosome is fragmented. Random pieces of it may be attached to viral DNA and be incorporated in a new virus assembly. General transduction involves virulent viruses that will go into the lytic cycle.*
   In restricted transduction, the viral nucleic acid becomes incorporated into the bacterial host chromosome. Restricted transduction involves temperate (non-virulent) viruses that will go into the lysogenic cycle. When the virus goes into the lytic cycle and the host's chromosome is broken, the bacterial DNA that might be attached to the viral DNA are those pieces adjacent to the virus DNA. Specific pieces of the host DNA, rather than random pieces, are incorporated.
3. *Restricted transduction would be more useful because you could predict which pieces of the bacterial DNA would be carried by a given virus. It would depend where on the host chromosome the virus became incorporated.*
4. *Answers will vary.*

Viruses as Vectors

Resource Materials

Supplementary readings for Part 1. Structure, Function and Life Cycles

Butler, P.L.G. & Klug, A. (1978, November). The assembly of a virus. *Scientific American.*

Campbell, A.M. (1976, June). How viruses insert their DNA into the DNA of the host cell. *Scientific American.*

Gallo, R.C. (1986, December). The first human retrovirus. *Scientific American.*

Simons, K., Garoff, H. & Helenius, A. (1982, February). How an animal virus gets into and out of its host cell. *Scientific American.*

Temin H.M. (1972, January). RNA-directed DNA synthesis. *Scientific American.*

Varmus, H. (1987, September). Reverse transcription. *Scientific American.*

Supplementary readings for Part 2: Transduction

Hapgood, F. (1987, November). Viruses emerge as a new key for unlocking life's mysteries. *Smithsonian.*

Moses, P.B. & Chua, N. (1988, April). Light switches for plant genes. *Scientific American.*

Ptashne, M., Johnson, A. & Palo, C. (1982, November). A genetic switch in a bacterial virus. *Scientific American.*

Sonea, S. (1987, July). Bacterial viruses, prophages and plasmids reconsidered. *Annals of the New York Academy of Sciences, Endocytobiology III.*

Weaver, R. (1984, December). Changing life's genetic blueprint. *National Geographic.*

Viruses as Vectors

STUDENT PAGE 1

Part I. Virus Structure, Function and Life Cycles

Background Reading

Viruses can serve as vehicles to carry cellular DNA from one host to another in nature as well as through genetic engineering. In order to understand how this can be accomplished, it is necessary to understand the structure of viruses and the processes by which viruses infect cells. Since the best studied systems and those most useful to the genetic engineer are bacterial, this discussion will focus on bacteriophages, the viruses that infect bacteria.

Structurally, viruses consist of a molecule of nucleic acid (which, depending on the specific virus, may be DNA or RNA, single or double stranded, circular or linear) enclosed in a capsid, or protein coat. In addition, some viruses (mainly animal viruses) have an envelope made of lipids and glycoproteins surrounding the capsid. The nucleic acid behaves as do cellular chromosomes in acting as the genetic "blueprint," and the capsid protects this "blueprint" as it is passed from one host cell to another. The capsid proteins or envelope glycoproteins also recognize and bind to specific receptor molecules on the host cell's surface, so that viruses only attach to cells whose receptors are recognized by the viral protein.

Since viruses contain no cytoplasm or organelles to synthesize new molecules or to generate energy, they must invade a cell and commandeer that cell's enzyme systems and organelles. New viruses are made from the viral nucleic acid "blueprint" using the host's energy sources (such as ATP), raw materials (such as nucleotides and amino acids) and also the cell's metabolic machinery. For this reason, viruses have been called the ultimate parasite!

The viral nucleic acid, or chromosome, always codes for the virus coat proteins as well as for some enzymes. One type of enzyme forces the bacterial host cell to replicate the viral chromosome at a much faster rate than it does its own chromosome. Another type enables the newly formed virus particles to break open (lyse) the host cell, killing it and escaping to infect other host bacteria.

The composition of a virus particle's protein determines whether and where a virus will attach to the membrane of a host cell. Once the virus has attached, it makes a hole in the membrane, and the viral nucleic acid is injected into the host cell.

After a virus penetrates the bacterium, there are two choices:

1. Lytic Cycle. The virus acts as a "virulent phage," taking over the host cell's machinery and forcing it to make new viruses, then killing the bacterium as the newly formed viruses escape.

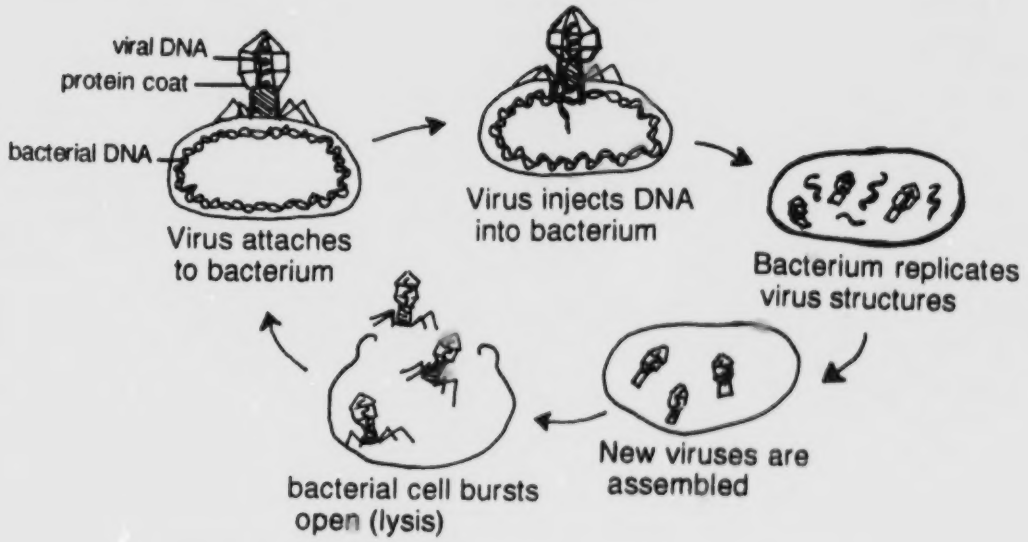
2. Lysogenic Cycle. The virus acts as a "nonvirulent (temperate) phage" and becomes integrated into the host bacterium's chromosome. Viruses that become part of the bacterial chromosome in this way are called *prophages*. The viral nucleic acid replicates each time the host chromosome does so that each new bacterium has a chromosome that contains the viral nucleic acid as well as its own. The host cell is not killed at the time of infection. This host cell is immune to further infection by that virus. Under certain conditions (such as x-rays, ultraviolet light or other agents that damage DNA),the prophage virus may break out and begin a lytic cycle.

Viruses as Vectors

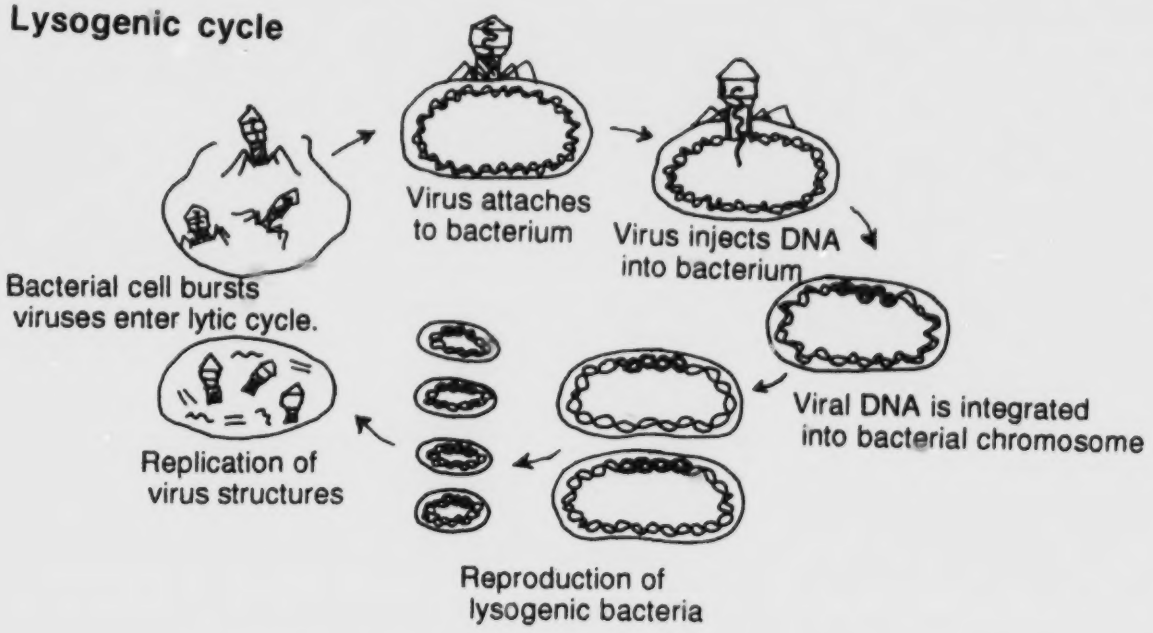
STUDENT PAGE 2

Virus Life Cycles

Lytic cycle



Lysogenic cycle


Virus attaches to bacterium
Virus injects DNA into bacterium
Bacterial cell bursts viruses enter lytic cycle.
Viral DNA is integrated into bacterial chromosome
Replication of virus structures
Reproduction of lysogenic bacteria

Adapted from *Biology: An Everyday Experience,* Merrill Publishing Company.

Viruses as Vectors

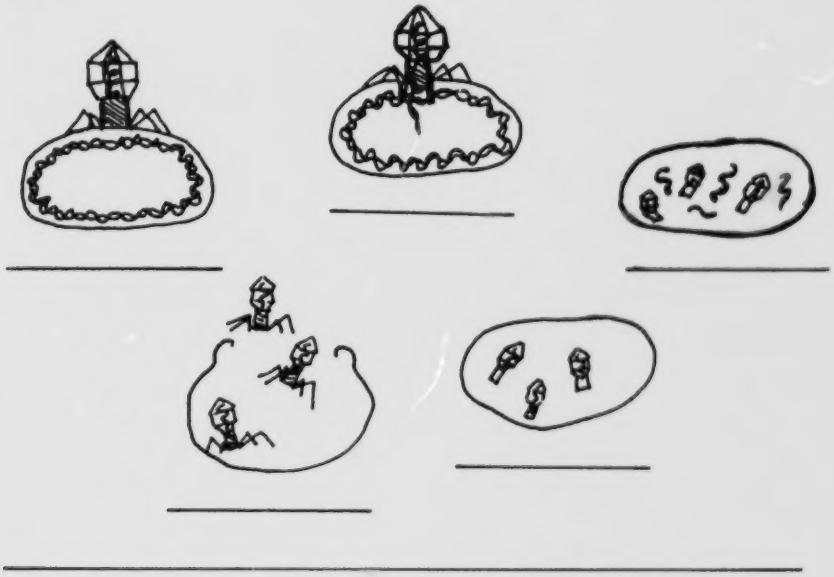
STUDENT PAGE 3

Part 1. Virus Structure, Function and Life Cycles

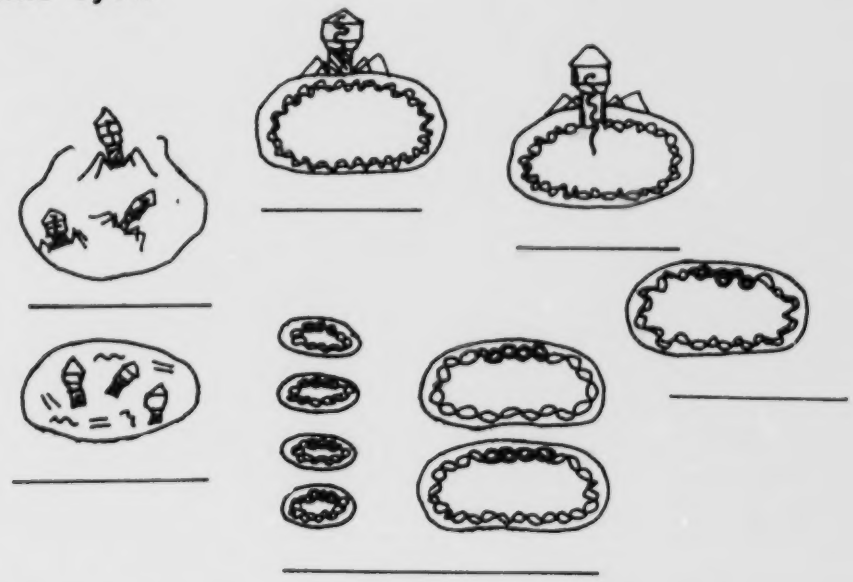
Worksheet

Use the diagrams below to check your understanding of the lytic and lysogenic cycles. Label the structures and the stages.

Lytic cycle



Lysogenic cycle



Adapted from *Biology: An Everyday Experience,* Merrill Publishing Company.

Viruses as Vectors

STUDENT PAGE 4

Part 1. Virus Structure Function and Life Cycles

Questions

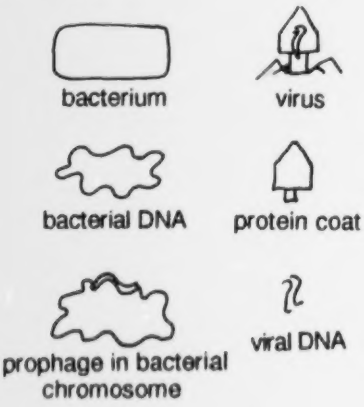
1. Compare a lytic infection with a lysogenic infection - include the activity of the virus and the effect on the host cell.

2. Use the following key to answer questions i-iii:
   A. Lytic virus
   B. Lysogenic virus
   C. Both

   i. The host's DNA is destroyed by the virus.
   ii. Viral proteins are synthesized on the host ribosomes.
   iii. The viral genome is incorporated into the host's genome.

3. Hypothesize what characteristics of viruses make them particularly useful as vectors in gene transfer experiments.

4. Below are symbols of the virus and bacterium at various stages. Use these symbols to make eight drawings, one each of the listed lettered steps (a-h) which describe the infection of *E. coli* by a bacteriophage. Label each structure in your drawings.



a. Virus attaches to bacterial cell wall.
b. Virus injects nucleic acid into the bacterium.

Lytic cycle

c. Virus takes over bacterial metabolism and causes synthesis of new viral proteins and new viral DNA.
d. Viral protein and viral DNA assemble into complete viruses.
e. An enzyme coded for by the viral DNA breaks open the bacterium, and the new viruses are released.

Lysogenic cycle

f. Viral DNA becomes incorporated into the bacterial chromosome. (The virus is called a prophage while so incorporated.)
g. Bacterium replicates, and viral DNA replicates when the bacterial DNA does.
h. Eventually conditions (X-rays, uv light or other agents that damage nucleic acids) may cause the prophage to break loose from the host chromosome, and the virus enters the lytic cycle.

For Further Research

A. How does the Semiliki Forest virus (an animal virus) infection compare with the lytic and lysogenic bacteriophage infection?

B. Viruses can be either DNA or RNA viruses. Discuss characteristics of each.

C. Describe the unique genetic ability possessed by retroviruses. How is that ability used in genetic engineering?

Viruses as Vectors

STUDENT PAGE 5

Part 2. Virus Transduction

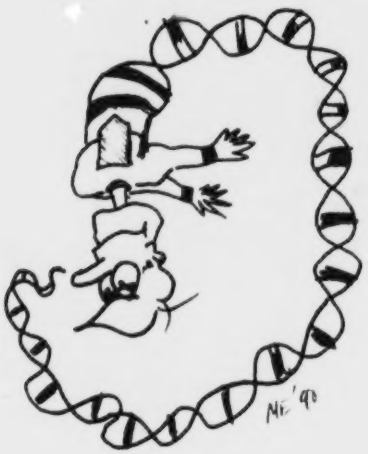
Background Reading

Transduction occurs when bacterial DNA is transferred from one cell to another by means of a virus. There are two types of transduction: **General transduction,** which occurs during the lytic cycle; **restricted transduction** which takes place in the lysogenic cycle.

In each kind of transduction, fragments of bacterial DNA are caught up in a newly formed virus and are carried to a new bacterial cell. There they may become part of the bacterial chromosome. This results in a recombinant bacteria. Scientists have copied this natural process to genetically engineer new types of bacteria. Viruses are used as "gene taxis" to carry DNA from one bacteria to another.

In the lytic cycle, **general transduction** occurs when a virus injects its DNA into a host bacterial cell and forces the bacteria to make new viruses. The bacterial chromosome often is broken, and random fragments may accidently become incorporated in newly formed protein coats along with the viral DNA. These new viruses go on to infect another bacteria, inadvertently transferring bacterial genes along with their own DNA.

In the lysogenic cycle, viral DNA is already incorporated into the bacterial chromosome and breaks loose only when something happens to damage the bacterial DNA, such as ultraviolet light. When **restricted transduction** occurs, the viral DNA breaks loose and takes a piece of bacterial DNA with it. This is incorporated into the new virus. This is always the same piece of bacterial DNA, since the viral DNA always enters the bacterial DNA at a particular sequence. Therefore, transduction is *restricted* to that part of the bacterial DNA attached to the viral DNA. When the new virus goes into the lysogenic cycle and combines with a bacterial chromosome, that particular part of the previous bacteria's DNA will be included in the current host's DNA.



Viruses as Vectors
STUDENT PAGE 6

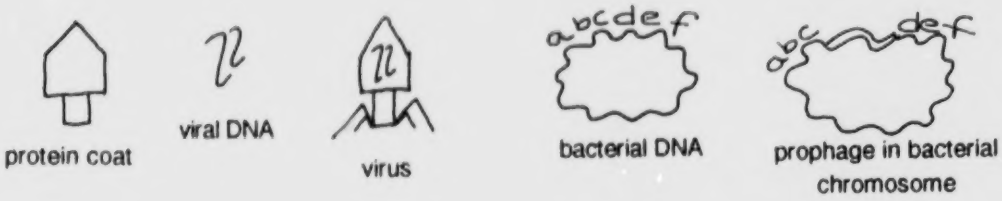
Part 2. Virus Transduction
Worksheet

Diagram instructions

1. Complete the transduction diagrams on the following pages. Each rectangle represents a bacterium and you are to choose appropriate structures from the symbols presented below to illustrate the stage presented. **NOTE:** The letters, a b c d e f, on the bacterial chromosome simply represent adjacent sections. If you use these letters, you can follow the fate of the various sections through the fragmentation of the chromosome and its subsequent linking with the viral DNA and/or the new bacterium's chromosome.

Use the following labels on your diagrams as appropriate:

bacterial chromosome
bacterium
linked viral-bacterial DNA
virus
prophage
recombinant cell
viral protein coat
viral nucleic acid



NOTE: Use a colored pen or pencil to differentiate between viral nucleic acid and bacterial nucleic acid. You are differentiating between original bacterial cell DNA and new host cell bacterial DNA to illustrate that a recombinant bacterium has been produced by the virus carrying "old" bacterial DNA into a new bacterial cell.

Viral DNA ..... Red
DNA from original bacterial cell ..... Black
DNA from new host bacterial cell ... Green

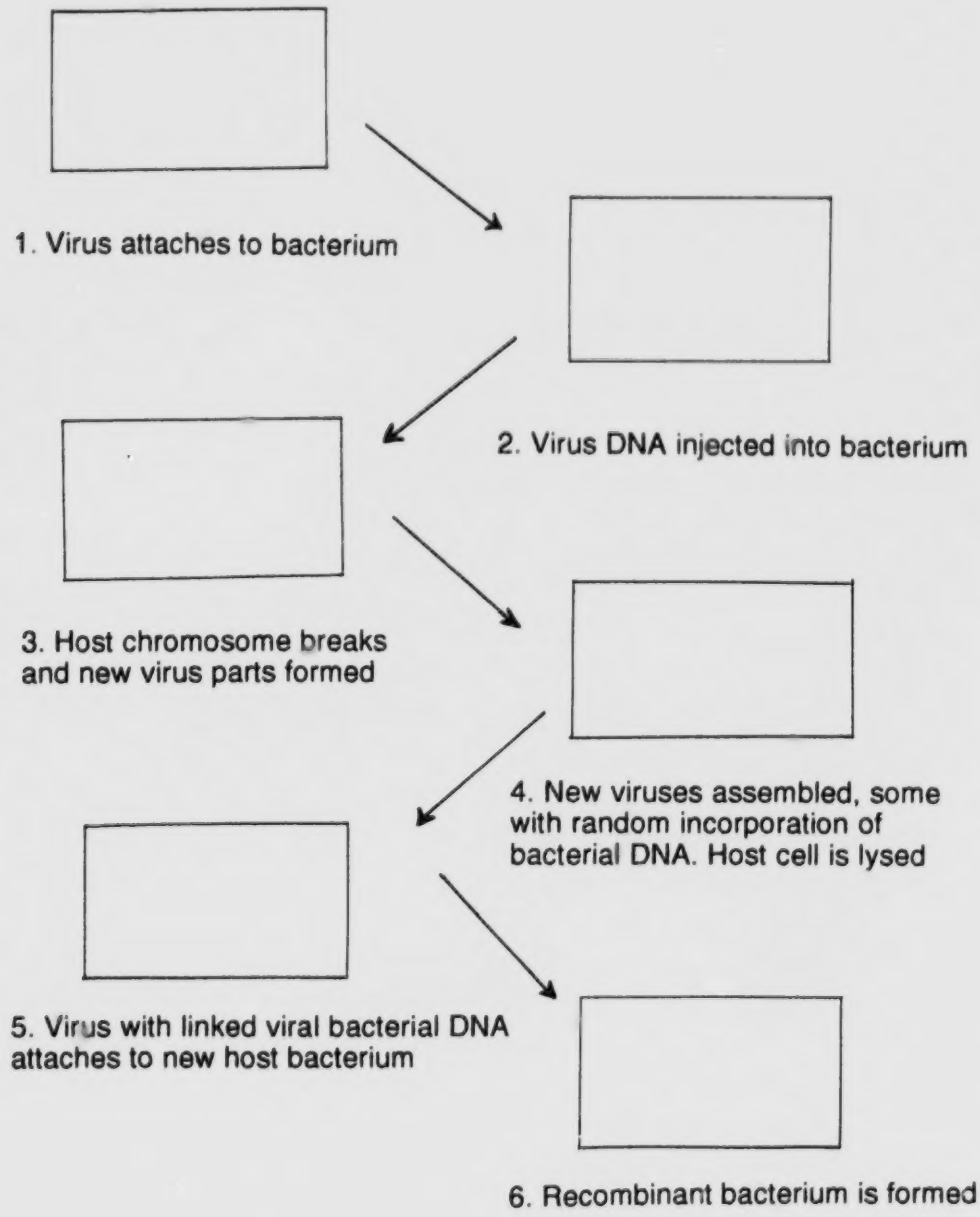
Viruses as Vectors

STUDENT PAGE 7

Part 2. Virus Transduction

Diagram of General Transduction

Label the diagram below after reading the instructions.



1. Virus attaches to bacterium

2. Virus DNA injected into bacterium

3. Host chromosome breaks and new virus parts formed

4. New viruses assembled, some with random incorporation of bacterial DNA. Host cell is lysed

5. Virus with linked viral bacterial DNA attaches to new host bacterium

6. Recombinant bacterium is formed

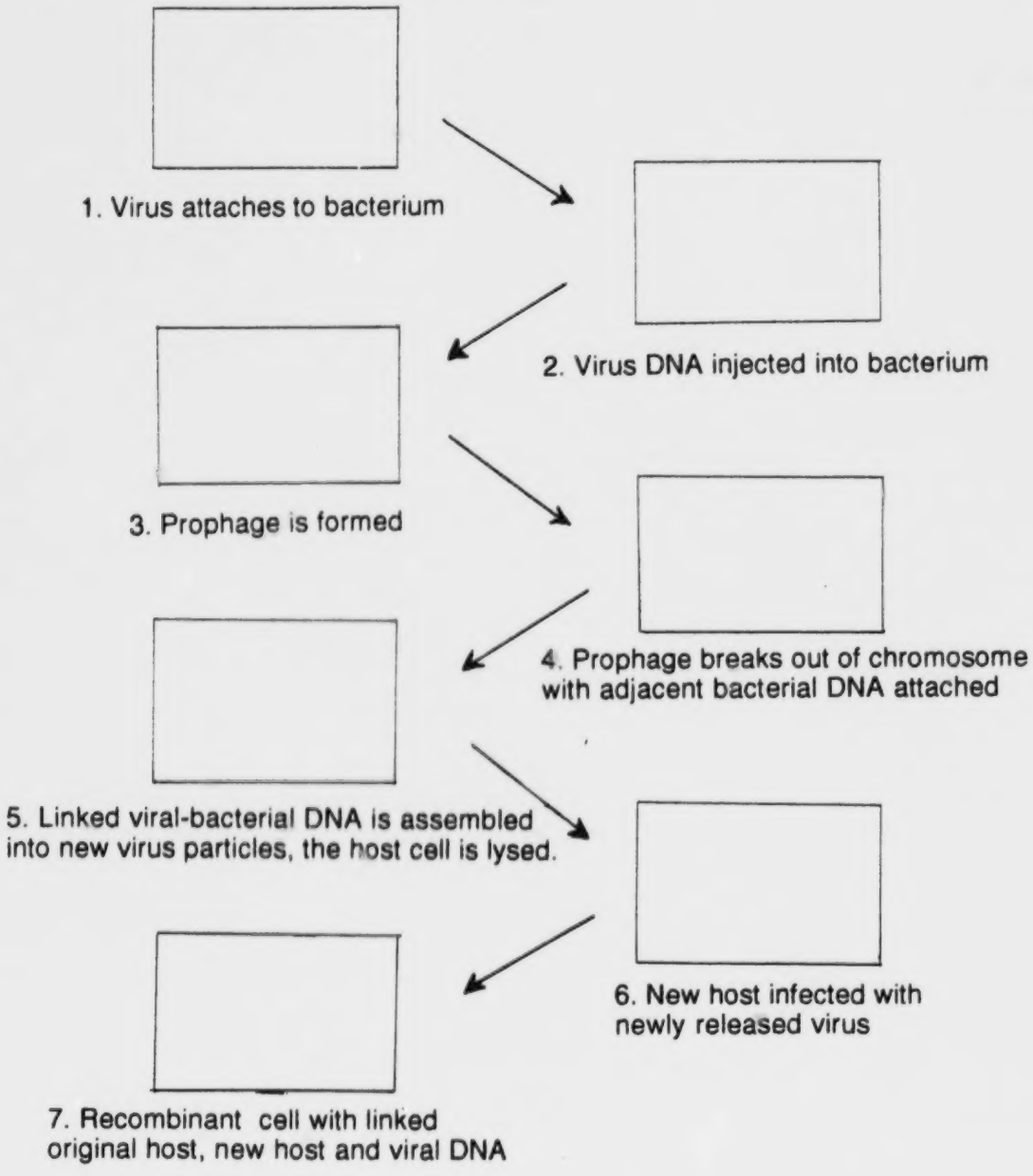
Viruses as Vectors

STUDENT PAGE 8

Part 2. Virus Transduction

Diagram of Restricted Transduction

Label the diagram below after reading the instructions.


1. Virus attaches to bacterium
2. Virus DNA injected into bacterium
3. Prophage is formed
4. Prophage breaks out of chromosome with adjacent bacterial DNA attached
5. Linked viral-bacterial DNA is assembled into new virus particles, the host cell is lysed.
6. New host infected with newly released virus
7. Recombinant cell with linked original host, new host and viral DNA

Viruses as Vectors

STUDENT PAGE 9

Part 2. Virus Transduction

Questions

Answer the following questions after you have completed the diagram pages.

1. How does transduction serve as a means of genetic recombination?

2. Compare and contrast general and restricted transduction.

3. From your knowledge of cloning, do you think general or restricted transduction would be of more use to the genetic engineer?

4. Describe an experiment in which transduction has been used.

Research Restated

Ability level ..... Honors, AP biology
Class periods required ..... 1 + 2 homework assignments

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Introduction

This activity uses two articles to teach both scientific content and good writing techniques. Students read a primary research article and then write a popularized article from it, aiming for scientific accuracy, simplification and level of interest. They then compare their writing with a published popularized article. The articles chosen for this lesson discuss the transfer of genetic material between species.

Objectives

After this activity, students should be able to:

1. Assess a popularized research article in terms of accuracy, simplification and interest.
2. Write a popularized article from a piece of primary research.
3. Describe the transfer of genetic material across distant species by conjugation.

Materials

- The articles:
  Heinemann, J.A. & Sprague, G.F. Jr. (1989). Bacterial conjugative plasmids mobilize DNA transfer betweeen bacteria and yeast. *Nature, 340*, 205-209.

  Booth, W., Gladwell, M. & Sawyer, K. (1989, July). Science notebook: Crossing distant species. *The Washington Post*, p. A2.

Teacher Preparation

Read the articles and make copies for the students.

Teaching Tips

Look for other pairs of research and popular articles and discuss with the class the way that science writers translate research findings into poular articles.

Procedure

1. Students read the primary research article.
2. Students attempt to write an article for a hypothetical newspaper or popular magazine based on the primary research.
3. Students compare their article to the one printed in *The Washington Post*.

Answers to Student Questions

Will vary with the student.

Research Restated

STUDENT PAGE

Activity Sheet

Genetic Transfer Across Species

Introduction

This activity will help you improve your writing skills as well as teach you some science. You will analyze what skills are necessary to "translate" a primary research article into a popularized article.

1. Read the following primary research article:

   Heinemann, J.A. & Sprague, G.F. Jr. (1989). Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. *Nature ,340,* 205.

2. Write an article for a hypothetical newspaper (or magazine) based on the primary research you just read. Keep in mind these goals:
   a. Accuracy of scientific content
   b. Readability (simplification of content)
   c. Level of interest generated (the enjoyment factor)

3. Compare article you wrote to "Science notebook: Crossing distant species" from *The Washington Post*. Give your article and the one from *The Post* a grade based on accuracy, readability and interest level. Justify your choice of grade with specific examples from the articles.

Questions

1. List attributes that you feel are necessary for successful popularization of primary research science articles.

2. Discuss the similarities and differences between the transfer of genetic material by sperm and the process of conjugation.

3. Comment on the evolutionary significance of the two processes.

Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast

Jack A. Heinemann* & George F. Sprague Jr

Institute of Molecular Biology* and Department of Biology, University of Oregon, Eugene, Oregon 97403, USA

Conjugative plasmids of *Escherichia coli* can mobilize DNA transmission from this bacterium to the yeast *Saccharomyces cerevisiae*. The process shares some of the features of conjugation between bacteria and could be evolutionarily significant in promoting trans-kingdom genetic exchange.

TRANSMISSION of genetic information between distant taxonomic groups could create organisms with unique evolutionary potential. One well-studied example of such gene transmission occurs between the bacterium *Agrobacterium tumefaciens* and certain plant species (for review, see ref. 1). It seems that DNA transfer from *A. tumefaciens* to plants is mechanistically similar to bacterial conjugation[2,3]. Most strikingly, conjugative plasmids can substitute for tumour-inducing plasmid functions necessary for transmission of DNA to plants, provided that certain tumorigenic-encoded virulence functions that contribute to DNA transfer are also present[4]. Here we investigate whether bacterial conjugation could provide a general mechanism for inter-kingdom genetic exchange using derivatives of two classes of bacterial conjugative plasmids: broad-host-range plasmids, such as R751, which promote conjugation between a large number of bacterial species, including between Gram-negative and Gram-positive species[5-7], and the F plasmid (fertility factor), which confers a limited-host-range phenotype, its conjugation being limited to *E. coli* and related Enterobacteriaceae[8]. Here we show that both broad-host-range and limited-host-range conjugation plasmids can transmit DNA from bacteria to yeast.

Transfer of plasmids between bacteria by conjugation requires cell contact and DNA metabolism[9-12]. Transmission to the new host also requires that the plasmid should replicate, either autonomously or by association with a host replicon. The donor cell in a conjugal relationship contains a self-transmissible plasmid which encodes the capacity to carry out the transfer functions. The plasmid *tra* locus encodes cell-surface components as well as mobilization proteins (*mob* gene products) which promote DNA transfer by interaction with a *cis*-acting sequence termed the origin of transfer (*oriT*). We use representatives of three different plasmid groups: R751, ColE1 and the fertility factor F. For simplicity, suffixes are used to denote the origin

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of conjugation functions: for example, *mob*-R and *oriT*-R refer to the *mob* and *oriT* functions of the R751 group respectively (Table 1).

DNA transmission from bacteria to yeast

To determine whether plasmids can be transmitted from bacteria (*E. coli*) to yeast (*S. cerevisiae*) by conjugation, we have used two bacterial strains, one containing both a self-transmissible plasmid and a plasmid that can replicate and be selected in yeast, and the other containing only the yeast plasmid. The plasmid pDPT51, a derivative of the broad-host-range plasmid R751, contains *mob*-R and *oriT*-R for self-transmission and also the *mob*-C region from ColE1 (Table 1). Thus, pDPT51 can mobilize *in trans* other plasmids, such as pBR322, that contain the *oriT* region from ColE1 (*oriT*-C, or the *bom* site[9]). We used the yeast plasmid YEp13 (Table 1), which is a yeast-bacteria shuttle vector derived from pBR322. YEp13 carries the yeast *LEU2* gene and a segment of the yeast 2μ plasmid, allowing selection and replication of the plasmid in yeast.

We tested *E. coli* cells containing both plasmids for the ability to transmit plasmid DNA to yeast cells and to other *E. coli* strains using a standard protocol for bacterial conjugation (see Table 2 legend). When donor bacteria are mixed with *leu2*⁻ yeast cells, Leu⁺ yeast are detected at a frequency of 3×10^{-7} Leu⁺ cells per recipient (Table 2*a*). We confirmed that the Leu⁺ yeast cells contained YEp13 by restriction-endonuclease analysis of plasmid DNA recovered from these cells. Although we presume that pDPT51 was also transferred to yeast, we did not detect its transmission because it lacks a replication sequence for propagation in yeast.

The frequency of transmission of YEp13 ranges from 3×10^{-8} to 5×10^{-5} Leu⁺ cells per recipient and can be as high as 7×10^{-2} Leu⁺ cells per donor, depending partly on the ratio of donor to recipient cells in the mating assay (Tables 2 and 3). In bacterial conjugation experiments, transconjugant bacteria arose at a frequency of $\sim 3 \times 10^{-1}$ transconjugants per recipient (Table 2). Initially we made no attempt to optimize the ratio of bacteria to yeast in the mating assay, but later found that an excess of bacterial cells reduced the frequency of DNA transmission, which has been found for conjugation between bacteria[9]. The donor:recipient ratios used in each experiment are given in Tables 2 and 3.

To see whether the appearance of Leu⁺ yeast cells was dependent on the conjugative plasmid, *E. coli* carrying only YEp13 was mixed with yeast. No Leu⁺ yeast was observed ($< 1 \times 10^{-9}$ Leu⁺ cells per recipient; Table 2*a*), indicating that DNA transmission was dependent on a conjugation plasmid. We therefore

TABLE 1 Plasmid functions

Plasmid	Conjugation functions	Selectable markers* in bacteria	in yeast	Refs
R751	*tra*-R, *mob*-R, *oriT*-R	Tp′	none	(19)
pDPT51	*tra*-R, *mob*-R, *oriT*-R, *mob*-C	Tp′, Am′	none	(20)
pDPT51 LEU2	*tra*-R, *mob*-R, *oriT*-R, *mob*-C	Tp′, Am′	*LEU2*	This work
YEp13	*oriT*-C	Tc′, Am′	*LEU2*	(21)
YEp24	*oriT*-C	Tc′, Am′	*URA3*	(22)
pAL2	none	Cm′	*LEU2*	This work
pACYC184	none	Cm′ Tc′	none	(15)
pFLEU2	*tra*-F, *mob*-F, *oriT*-F	Am′	*LEU2*	This work
pRS2405	*tra*-F, *mob*-F, *oriT*-F	Am′	none	(23)

Plasmids R751, pDPT51, YEp13, YEp24, pACYC184, and pRS2405 have been described (refs 15, 19–23). Plasmids R751 and pDPT51 are broad-host-range conjugative plasmids of the P incompatibility group. Plasmid pRS2405 is a limited-host-range plasmid of the F incompatibility group. pAL2 was constructed by cloning an *Xho*I–*Hin*dIII fragment that contains *LEU2* and 2μ sequences from YEp13 into the *Sal*I and *Hin*dIII sites of pACYC184, resulting in the deletion of the *tet*′ gene of pACYC184. pFLEU2 and pDPT51:*LEU2* were constructed in multiple steps. First, the *Xho*I–*Hin*dIII 2μ, *LEU2* fragment of YEp13 was cloned into the *Sal*I–*Hin*dIII sites of pUC12 (ref. 24). The 2μ, *LEU2* fragment was then cloned as an *Eco*RI (from the pUC12 polylinker)–*Hin*dIII fragment into the *Eco*RI and *Hin*dIII sites of pBR322 (ref. 25). In the resulting plasmid, the 2μ, *LEU2* segment is flanked by *Bam*HI sites, one from the pUC polylinker and one in the *tet*′ gene of pBR322. To create pFLEU2, the *Bam*HI fragment was inserted into the *Bam*HI site of pRSF2405. To create pDPT51:*LEU2*, the *Bam*HI fragment was first cloned into the *Bam*HI site of pNK1207 (ref. 26), a derivative of pBR333. The resulting plasmid was integrated into pDPT51 by recombination *in vivo* to yield pDPT51:*LEU2*. All plasmids other than R751, pDPT51, pACYC184 and pRS2405 contain yeast 2μ-plasmid sequences which permit automomous replication in yeast.

* The plasmids contain genes which confer resistance to antibiotics in bacteria. Tp′ indicates that bacteria harboring the plasmid are resistant to trimethoprim; Am′, resistance to ampicillin; Tc′, resistance to tetracycline; and Cm′, resistance to chloramphenicol. In addition, some plasmids contain the yeast *LEU2* or *URA3* genes. These plasmids can be selected in yeast because they complement nutritional auxotrophies conferred by mutations in the chromosomal *LEU2* or *URA3* genes.

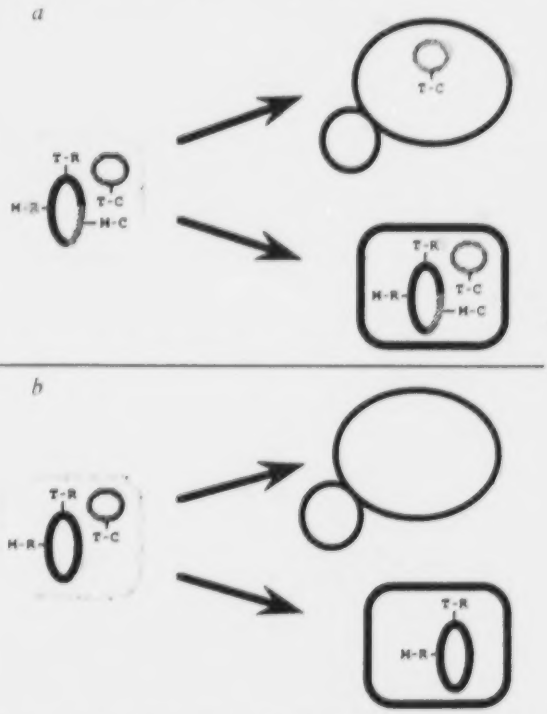

FIG. 1 Representation of the requirement for appropriate mobilization functions for plasmid transmission to bacteria and to yeast, as interpretated from results presented in Table 3, *a*1 and *a*2. The large rounded squares represent bacteria; donor bacteria are depicted with stippled borders and recipient bacteria with solid borders. The large budded circle represents yeast. Circles and ellipses within the cells represent plasmids. The ellipse with the solid border is plasmid R751. Plasmid pDPT51 is a derivative of R751 that carries the *mob*-C region (depicted by wide cross-hatching) from ColE1. The circle with the horizontally-striped border represents the bacteria–yeast shuttle vector YEp13. The *mob* and *oriT* functions present on the plasmids are represnted by abbreviations M-R, *mob*-R; T-R, *oriT*-R; M-C, *mob*-C; T-C, *oriT*-C. *a*, The *mob*-C function of pDPT51 acts at the *oriT*-C of YEp13 and promotes its transmission to both bacteria and yeast. The *mob*-R function of pDPT51 acts at the *oriT*-R present on the same plasmid and promotes its transmission to bacteria. In principle, pDPT51 may also be transferred to yeast, but the plasmid lacks functions that permit replication and selection in yeast, so transfer cannot be detected. *b*, Plasmid R751 is transmitted to bacteria by means of its *mob*-R and *oriT*-R functions. However, YEp13 is not transmitted to either bacteria or yeast because no *mob*-C function is present in the donor bacterium. Again, R751 may also be transferred to yeast but this is undetectable.

TABLE 2 Physical requirements for plasmid transfer

Experiment (plasmids carried by donor)	Recipient*	Treatment	Transconjugant cells per ml	Donor recipient ratio	Transconjugant cells per recipient
a					
YEp13	Y	none	0	30 1	$< 1 \times 10^{-9}$
pDPT51, YEp13	Y	none	42	6 1	3×10^{-7}
b					
None	Y	YEp13 DNA	0	40 1	$< 5 \times 10^{-9}$
pDPT51	Y	YEp13 DNA	0	30 1	$< 1 \times 10^{-9}$
pDPT51, YEp13	Y	none	73	6 1	1×10^{-7}
No bacteria	Y	YEp13 DNA	0	—	$< 2 \times 10^{-9}$
c					
pDPT51, YEp13	Y	$CHCl_3$	0	—	$< 5 \times 10^{-10}$
pDPT51, YEp13	Y	$CHCl_3$ buffer	15	3 1	2×10^{-7}
pDPT51, YEp13	Y	none	11	1 2	3×10^{-8}
d					
pDPT51, YEp13	Y	pellet	3×10^{-2}	1 300	3×10^{-6}
pDPT51, YEp13	B	pellet	2×10^{-1}	1 30	2×10^{-1}
pDPT51, YEp13	Y	supernatant	0	—	$< 2 \times 10^{-8}$
pDPT51, YEp13	B	supernatant	0	—	$< 2 \times 10^{-7}$
e					
pDPT51, YEp13	Y	separated by filter	0	—	$< 5 \times 10^{-9}$
pDPT51, YEp13	B	separated by filter	0	—	$< 5 \times 10^{-10}$
pDPT51, YEp13	Y	combined on filter	1×10^{-4}	1 30	5×10^{-6}
pDPT51, YEp13	B	combined on filter	2×10^{-5}	1 2	3×10^{-1}

Donor and recipient cells were prepared for conjugation by a procedure adapted from Sistrom *et al.*[27]. Exponential cultures of donor bacteria growing in medium selective for plasmid maintenance were collected by centrifugation, washed with TNB (0.05 M Tris, pH 7.6, 0.05% NaCl) and resuspended in TNB (10^{10}–10^{11} cells per ml). Similarly, exponential cultures of recipient cells growing in rich medium (LBH (1% tryptone, 0.5% NaCl, 0.5% yeast extract, 1 mM NaOH)[28] for bacteria, or YEPD (2% peptone, 1% yeast extract, 2% glucose)[29] for yeast) were collected and resuspended in TNB (10^{10} cells per ml). Donor and recipient were mixed in the ratios indicated and plated as described below. Reported results are from representative experiments. Experiments *a*, *b* and *c* used bacterial strain RR1 (*proA2 leuB6 hsdS20 thi ara14 galK2 xyl5 mtl1 lacY1 supE44 rpsL20*; ref. 25) as the donor, and yeast strain DC5 (*MATa leu2-3 leu2-112 his3 gal2 can1*) as the recipient. Experiments *d* and *e* used bacterial strain SB21 (*hsdS leuB6 thi*, a derivative of C600; ref. 30) as the donor. SY1229 (*MATa leu2-3 leu2-112 his3 gal2 can1 ura3*) as the yeast recipient, and RR1 as the bacterial recipient. Two other yeast strains have also served as recipients, one of which is only distantly related to DC5 and SY1229. Experiment *a* established that the formation of Leu$^+$ yeast was dependent on a bacterial conjugation plasmid. The mixture of donor and recipient was plated directly on supplemented yeast minimal medium (SD (10.67% yeast nitrogen base, 2% glucose) + his (20 μg ml^{-1} L-histidine); ref. 29). The number of transconjugants per ml was calculated on the basis of the volume of the mixture applied to the solid medium. No reversion to Leu$^+$ was detected. Incubation of the conjugation mixture on non-selective medium before plating gave comparable frequencies of Leu$^+$ transconjugants. Experiment *b* demonstrated that extracellular YEp13 DNA did not yield Leu$^+$ yeast. The donor–recipient mixture was preincubated on solid non-selective medium for 5–12 hours, resuspended in TNB, and plated on SD + his. The number of transconjugants per ml was calculated on the basis of their concentration in this final suspension of TNB. The concentration of YEp13 DNA in the mixture was 6 μg ml^{-1}. Experiment *c* showed that the formation of Leu$^+$ yeast was dependent on viable donor cells. The donor–recipient mixture was treated as in experiment *b*. Experiment *d* demonstrated that the formation of Leu$^+$ yeast was not caused by an extracellular diffusible factor. Pelleted donor cells or the supernatant of the donor culture was mixed with the yeast recipient and plated directly on SD + his + ura (20 μg ml^{-1} uracil). All bacterial matings (also in Table 3) were first plated on LBH to allow expression of the antibiotic-resistant phenotype. After 5 h the cells were resuspended and plated on OMBG (minimal agar plates; ref. 31) plus proline (20 μg ml^{-1}), leucine (20 μg ml^{-1}) and trimethoprim (Tp) (200 μg ml^{-1}) and tetracyclin (Tc) (20 μg ml^{-1}). The frequency of transconjugant bacteria reflects the number of bacteria that received both plasmids. The same frequency was observed when transmission of only YEp13 was monitored. Experiment *e* confirmed that the formation of Leu$^+$ yeast required cell-to-cell contact. The mixture was preincubated on solid non-selective medium for 5–12 h and then plated on SD + his + ura. The bacterial matings were performed as in experiment *d*.

* Y indicates that the recipient organism was yeast, B indicates that the recipient organism was bacteria.

refer to the Leu$^+$ yeast obtained by co-culture with bacteria as 'transconjugants'.

DNA is transmitted through conjugation

We used two different approaches to decide whether plasmid DNA can be transmitted from bacteria to yeast by a mechanism equivalent to bacterial conjugation: the first involved physically disturbing the conjugal environment so as to disrupt bacterial conjugation, and the second was to disrupt genetically the yeast–bacteria system and to determine whether transmission needed the plasmid loci necessary for conjugation between bacteria.

Physical requirements for DNA transmission. DNA transfer between bacteria by conjugation is distinguished from transduction or transformation by a dependence on cell contact, donor-cell viability and residence of the plasmid in the donor cell at the time of cell-to-cell contact. It was conceivable that the transmission of YEp13 to yeast occurs by a process unrelated to bacterial conjugation. For example, bacteria with conjugation plasmids may lyse more frequently or increase by other means the concentration of extracellular DNA in their vicinity. In four separate experiments we have demonstrated that the transmission of YEp13 DNA from bacteria to yeast has the same physical requirements as bacterial conjugation: (1) Exogenously added plasmid is not transmitted to yeast cells. Yeast cells were subjected to the conjugation protocol in the presence of more YEp13 DNA than would be released if all bacterial cells in the bacteria–yeast conjugation system had lysed. The 'mock' conjugations were performed with YEp13 alone, with YEp13 and plasmid-free bacteria, or with YEp13 and *E. coli* containing only the pDPT51 plasmid. Leu$^+$ yeast cells were not observed in any of these experimental combinations (Table 2*b*). The same yeast culture was mixed with *E. coli* cells containing both pDPT51 and YEp13 as a positive control, and Leu$^+$ transconjugant yeast were evident. We also demonstrated that extracellular DNA could not be responsible for the occurrence of Leu$^+$ yeast by adding DNaseI to the bacteria–yeast mixture, and we found no interference with DNA transmission (data not shown; ref. 13). (2) Viable donor cells are required. A culture of *E. coli* containing pDPT51 and YEp13 was split into three. The first sample was treated with chloroform to kill the cells, and the chloroform then removed[14]. The second sample was treated with buffer prewashed with chloroform in the same way as the first to test the efficiency of chloroform removal. The third sample was untreated. Leu$^+$ yeast cells were not recovered when the chloroform-treated cells were used as donors, but were with the buffer-treated or untreated cells (Table 2*c*). (3) DNA transmission does not involve an extracellular diffusible factor. A

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TABLE 3 Genetic requirements for plasmid transfer

Experiment (plasmids carried by donor)	Recipient*	Selection	Transconjugant cells per ml	Donor: recipient ratio	Transconjugant cells per donor
a1					
pDPT51 (*mob*-C, *mob*-R, *oriT*-R, Tp^r)	Y	Leu$^+$	5×10^{3}	1:1,000	7×10^{-2}
YEp13 (*oriT*-C, Tc^r, *LEU2*)	B	Tp^r	4×10^{6}	1:6,000	2
	B	Tp^r + Tc^r	5×10^{6}	1:6,000	3
	B	Tc^r	4×10^{6}	1:6,000	2
a2					
R751 (*mob*-R, *oriT*-R, Tp^r)	Y	Leu$^+$	0	1:100	$<2\times10^{-5}$
YEp13 (*oriT*-C, Tc^r, *LEU2*)	B	Tp^r	4×10^{6}	1:4,000	2
	B	Tp^r + Tc^r	0	1:4,000	$<2\times10^{-6}$
	B	Tc^r	0	1:4,000	$<2\times10^{-6}$
b1					
pDPT51 (*mob*-C, *mob*-R, *oriT*-R, Tp^r)	Y	Ura$^+$	1×10^{3}	5:1	3×10^{-5}
YEp24 (*oriT*-C, Tc^R, *URA3*)	Y	Leu$^+$	0	5:1	$<3\times10^{-7}$
pAL2 (Cm^r, *LEU2*)	B	Tp^r	2×10^{7}	1:6	6
	B	Tc^r	2×10^{6}	1:6	4×10^{-1}
	B	Cm^r	4×10^{4}	1:6	8×10^{-3}
b2					
pDPT51 (*mob*-C, *mob*-R, *oriT*-R, Tp^r)	Y	Leu$^+$	1×10^{2}	30:1	3×10^{-7}
pAL2 (Cm^r, *LEU2*)	B	Tp^r	3×10^{5}	3:1	3×10^{-1}
	B	Cm^r	8×10^{7}	3:1	3×10^{-3}
b3					
pDPT51 (*mob*-C, *mob*-R, *oriT*-R, Tp^r)	B	Tp^r	2×10^{6}	1:1	1×10^{-1}
pACYC184 (Cm^r)	B	Cm^r	0	1:1	$<5\times10^{-6}$
c1					
pFLEU2 (*mob*-F, *oriT*-F, *LEU2*)	Y	Leu$^+$	1×10^{2}	5:1	5×10^{-8}
c2					
pDPT51 *LEU2* (*mob*-R, *oriT*-R, *mob*-C, *LEU2*)	Y	Leu$^+$	1×10^{2}	3:1	1×10^{-7}
c3					
R751 (*mob*-R, *oriT*-R)	Y	Leu$^+$	0	6:1	$<3\times10^{-10}$
YEp13 (*oriT*-C, Tc^r, *LEU2*)	B	Tp^r	1×10^{6}	2:1	5×10^{-1}
	B	Tc^r	0	2:1	$<3\times10^{-9}$

Conjugation assays were performed as described for Table 2. During the course of these experiments it was discovered that a donor to recipient ratio of ~1:1,000 (10^4-10^5 donor cells per ml and 10^7-10^8 recipient cells per ml) resulted in a higher frequency of transmission than ratios closer to 1:1 (10^8 cells per ml of both types). Experiments reported are representative, not an average of multiple experimental results. The *a* set of experiments demonstrated that plasmid transmission was dependent on *mob* activity. The donor bacterial strain was SB21, the recipient yeast strain was SY1229, and the recipient bacterial strain was RR1. Leu$^+$ transconjugant yeast cells were selected by direct plating of the conjugation mixture on SD + his + ura (to select YEp13). Transconjugant bacteria were selected on OMBG + pro + leu + streptomycin (Sm; 100 μg ml^{-1}) medium which also contained either Tp alone (to select pDPT51 or R751) or both Tp and Tc (to select pDPT51 and YEp13). Transconjugant bacteria were also selected on LBH + Sm + Tc (to select YEp13). The *b* set of experiments established the specificity of the *mob*-*oriT* interaction. The donor bacterial strain was DH1 (*recA1 endA1 gyrA96 thi-1 hsdR17 supE44*; ref. 32), the recipient bacterial strain was HB101 (*recA* derivative of RR1; ref. 25), and the recipient yeast strain was SY1229. Transconjugant yeast cells were selected by direct plating on SD + his + ura (to select pAL2) or on SD + his + leu (to select YEp24). Transconjugant bacteria were selected by plating on OMBG + pro + leu + Sm + Tp (to select pDPT51); on LBH + Sm + Tc (to select YEp24); or on LBH + Sm + chloramphenicol (Cm; 12 μg ml^{-1}) (to select pAL2 or pACYC184). Although there was no pAL2 transmission to yeast in our experiment *b1*, in repeat experiments it occurred at a frequency of 3×10^{-7}. The *c* set of experiments demonstrated that derivatives of the F plasmid were capable of promoting DNA transfer to yeast. The donor bacterial strain was DH1 (*c1* and *c2*) or SB21 (*c3*); the recipient bacterial strain was HB101, and the recipient yeast strain was SY1229. Transconjugant yeast cells were selected by preincubation on solid non-selective medium for 5-12 h before plating on SD + his + ura (to select pFLEU2, pDPT51 LEU2, or YEp13). Transconjugant bacteria were selected by plating on OMBG + pro + leu + Sm + Tp (to select R751), or on LBH + Sm + ampicillin (Am; 100 μg ml^{-1}) (to select YEp13).

* Y indicates that the recipient organism was yeast; B indicates that the recipient organism was bacteria.

culture of *E. coli* containing pDPT51 and YEp13 was divided into supernatant and pellet fractions by filtration through a 0.45-μm pore Millipore filter and each fraction mixed with a yeast culture in a conjugation assay. Leu$^+$ transconjugant yeast cells were recovered from the mixture of pellet and yeast, but no Leu$^+$ transconjugants were recovered when the yeast cells were mixed with the supernatant fraction (Table 2*d*). (4) Cell contact is required for DNA transmission. Yeast and donor bacteria were applied to the surface of a 0.45-μm pore Millipore filter in two different orientations. In one orientation the yeast and bacteria were on the same side of the filter and in the other, the filter separated them. Leu$^+$ transconjugant yeast cells were recovered only when the yeast and bacteria were on the same side of the filter (Table 2*e*).

Transconjugant yeast cells were recovered only when the conjugation plasmid and YEp13 were present in a viable donor and when cell contact was allowed, thus demonstrating that the requirements were the same as for bacterial conjugation.

Genetic requirements for DNA transmission. We investigated whether DNA transmission required the conjugation functions *mob* and *oriT*. If the transmission of DNA from bacteria to yeast used the *mob* and *oriT* functions required for bacterial conjugation, then the transmission process should discriminate between plasmids using the same rules for *mob*/*oriT* interaction that characterize bacterial conjugation.

To test whether the ColE1 *mob* functions were necessary for transmission of YEp13 from bacteria to yeast, we compared the ability of *E. coli* cells containing either pDPT51 or R751 to transmit YEp13 to yeast. As noted above, pDPT51 contains *mob*-C, whereas its parent, R751, does not (Table 1); also, pDPT51 promotes transfer of YEp13 to yeast whereas R751 does not (Table 3, *a1* and *a2*; Fig. 1). Matings of these donor bacteria with other bacteria verifies that the R751 and pDPT51 conjugation systems are operative and have the properties expected. As shown in Table 3, *a1* and *a2*, *E. coli* containing pDPT51 and YEp13 can transmit both plasmids, whereas *E. coli* contain-

ing R751 and YEp13 can transmit only R751 to the recipient bacteria. We conclude that transmission of plasmid DNA from bacteria to yeast requires *mob* functions that are compatible with the *oriT* on the yeast plasmid.

The importance of *oriT* in DNA transmission from bacteria to yeast was tested by comparing transmission of *oriT*-C$^+$ and *oriT*-C$^-$ plasmids co-resident in a bacterial strain. We constructed a bacterial strain carrying pDPT51 (to provide *mob*-C functions) as well as *oriT*-C$^+$ or *oriT*-C$^-$ yeast plasmids (YEp24 and pAL2, respectively). As shown in Table 3, *b*1, transmission of the *oriT*-C$^+$ plasmid to yeast recipients is increased over that of the *oriT*-C$^-$ plasmid by a factor of ~100. Bacterial matings gave the same relative ratio of transmission of the two plasmids. We did not expect pAL2 to exhibit any transmission to either yeast or bacteria because its parent plasmid, pACYC184, is strongly *oriT*-C$^-$ and cannot be transmitted in bacterial matings (Table 3, *b*3; ref. 15). Evidently the *LEU2* or 2μ sequence in pAL2 has weak *oriT*-C activity or can undergo site-specific recombination with a plasmid present in the bacterial donor. The salient observation is, however, that the *oriT*-C$^+$ plasmid is transmitted at a much higher frequency than the *oriT*-C$^-$ plasmid.

We then established that differences in plasmid copy number or competition for transfer machinery could not be responsible for the different transmission frequencies of YEp24 and pAL2. DNA was extracted from the donor cells and used to transform *E. coli*[16]. Chloramphenicol- (pAL2) and tetracycline-(YEp24) resistant transformants were obtained at nearly equal frequencies (data not shown). Also, a donor containing only pDPT51 and pAL2 transmitted pAL2 to yeast and bacteria at essentially the same frequency as the bacterial strain containing pDPT51, pAL2 and YEp24 (compare Table 3, *b*1 and *b*2). Thus, efficient transmission of a plasmid from bacteria to yeast seems to require that the plasmid have an *oriT* that can be acted on by *mob* gene products present in the donor cell.

F factor mediates conjugation with yeast

The experiments described so far rely on cell contact and transfer functions encoded by the broad-host-range plasmid R751 and its derivative pDPT51, both of which promote conjugation between *E. coli* and many other bacterial species. The F plasmid, however, confers a limited-host-range phenotype. We tested whether the apparent limited-host-range of such a plasmid could be extended to include yeast by the incorporation of a sequence which would allow it to replicate autonomously in yeast. This approach has already shown that derivatives of F can be transmitted to *Pseudomonas aeruginosa* when they contain a sequence that confers autonomous replication in that species[8].

Plasmid pRS2405 (Table 1), a derivative of F (refs 9 and 10) that carries cell contact and transfer functions from F (*tra*-F) as well as *mob*-F and *oriT*-F, was engineered to carry the yeast *LEU2* gene and the 2μ replication sequence (pFLEU2; Table 1). To compare directly the transmission of the F plasmid to the R751-based plasmids, we constructed a derivative of pDPT51, pDPT51:LEU2, that could be monitored in yeast by virtue of *LEU2* and 2μ sequences. *E. coli* cells containing one or the other plasmid were able to conjugate with yeast to yield Leu$^+$ transconjugants (Table 3, *c*1 and *c*2), although transmission was less than that measured for YEp13 co-resident with pDPT51 (Table 3, *a*1). Both pFLEU2 and pDPT51:LEU2 (>50 kilobase pairs (kb)) are larger than YEp13 (11 kb) and so could either be transmitted less efficiently or have more difficulty in becoming established in yeast. Whatever the explanation, transmission of pFLEU2 and pDPT51:LEU2 reflects conjugal transmission to yeast, because we detected no transconjugant yeast cells in a control cross involving donor cells which could not transmit YEp13 (Table 3, *c*3).

Conclusions

We have found that bacterial plasmids that mediate conjugation between bacterial cells are also able to mediate plasmid transmission to the yeast *S. cerevisiae* by a process that seems, by our physical and genetic criteria, to be conjugation. Of the genetic functions required for conjugation between bacteria, we have shown that *mob* and *oriT* are necessary for bacteria–yeast conjugation. It remains to be determined whether *tra* functions required for cell-surface interactions in bacterial conjugation also participate in bacteria–yeast conjugation. Nonetheless, it is clear that two distinct cell-contact and transfer functions, one encoded by R751 and the other by F, and two *mob*/*oriT* systems, *mob*-C/*oriT*-C and *mob*-F/*oriT*-F, are capable of promoting DNA transmission from bacteria to yeast.

The conjugation functions encoded by the conjugative plasmids can apparently form contacts competent for DNA transfer with organisms separated by large evolutionary distances. Our results suggest that conjugation could occur between bacteria and animal cells and between bacteria and plant cells, even when the bacteria do not carry the specialised tumour-inducing plasmid. The conjugation system we have studied here provides a mechanism for the transfer of DNA between distantly related species. Such lateral transmission could be a significant factor in evolution and may help to explain discrepancies in phylogenic trees[17,18].

Finally, our system provides a rapid method, which in some cases could be superior to DNA transformation, for introducing plasmids into yeast and possibly into other organisms. Moreover, the system may provide a tractable way to investigate the molecular mechanism of transfer of DNA from *A. tumefaciens* to plant cells should it be possible to substitute yeast for the plant component. □

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1. Zambryski, P., Tempe, J. & Schell, J. *Cell* **56**, 193–201 (1989)
2. Stachel, S. E., Timmerman, B. & Zambryski, P. C. *Nature* **322**, 706–712 (1986)
3. Albright, L. M., Yanofsky, M. F., Leroux, B., Ma, D. & Nester, E. W. *J. Bact.* **169**, 1046–1055 (1987)
4. Buchanan-Wollaston, V., Passiatore, J. E. & Cannon, F. *Nature* **328**, 172–175 (1987)
5. Piffaretti, J. C., Arini, A. & Frey, J. *Molec. gen. Genet.* **212**, 215–218 (1988)
6. Brisson-Noel, A., Arthur, M. & Courvalin, P. *J. Bact.* **170**, 1739–1745 (1988)
7. Thiel, T. & Wolk, P. C. *Meth. Enzym.* **153**, 232–243 (1987)
8. Guiney, D. G. *J. molec. Biol.* **162**, 699–703 (1982)
9. Clark, A. J. & Warren, G. *Rev. Genet.* **13**, 99–125 (1979)
10. Ippen-Ihler, K. A. & Minkley, E. G. Jr. *Rev. Genet.* **20**, 593–624 (1986)
11. Willetts, N. & Wilkins, B. *Microbiol. Rev.* **48**, 24–41 (1984)
12. Thomas, C. M. & Smith, C. A. *Rev. Microbiol.* **41**, 77–101 (1987)
13. Trieu-Cuot, P., Carlier, C. & Courvalin, P. *J. Bact.* **170**, 4388–4391 (1988)
14. Jacob, A. E. & Hobbs, S. J. *J. Bact.* **117**, 360–372 (1974)
15. Chang, A. C. Y. & Cohen, S. N. *J. Bact.* **134**, 1141–1156 (1978)
16. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982)
17. Sneath, M. *J. molec. Evol.* **26**, 16–23 (1987)
18. Sneath, M. *Trends Genet.* **2**, 63–66 (1986)
19. Meyer, R. J. & Shapiro, J. A. *J. Bact.* **143**, 1362–1373 (1980)
20. Taylor, D. P., Cohen, S. N., Clark, W. G. & Marrs, B. L. *J. Bact.* **154**, 580–590 (1983)
21. Broach, J. R., Strathern, J. N. & Hicks, J. B. *Gene* **8**, 121–133 (1979)
22. Botstein, D. *et al. Gene* **8**, 17–24 (1979)
23. Ray, A., Cheah, K.-C. & Skurray, R. *Plasmid* **18**, 90–100 (1986)
24. Messing, J. & Vieira, J. *Meth. Enzym.* **101**, 20–78 (1983)
25. Bolivar, F. *et al. Gene* **2**, 95–113 (1977)
26. Huisman, O. & Kleckner, N. *Genetics* **116**, 185–189 (1987)
27. Sistrom, W. R. *et al. Arch. Microbiol.* **138**, 161–165 (1984)
28. Hershkowitz, I. & Signer, E. *J. molec. Biol.* **47**, 545–546 (1970)
29. Sherman, F., Fink, G. R. & Hicks, J. B. *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, New York, 1982)
30. Appleyard, R. K. *Genetics* **39**, 440–452 (1954)
31. Miller, J. H. *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, New York, 1972)
32. Low, B. *Proc. natn. Acad. Sci. U.S.A.* **60**, 160–167 (1968)

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SCIENCE NOTEBOOK

Crossing Distant Species

■ The ancient Greeks conjured visions of satyrs and centaurs and other unions of different species. Such chimeras, hybrids of very different species, have always been deemed impossible in reality. But new research suggests that a form of sex—the transfer of genes in a process known as conjugation—may actually be possible between organisms separated by a vast evolutionary distance.

In the current issue of Nature, University of Oregon researchers Jack A. Heinemann and George F. Sprague Jr. describe how the ubiquitous *Escherichia coli* bacteria, found in every creature's gut, can exchange genetic information with yeast cells, in much the same manner as it does with other bacterial cells. Bacteria and yeast are not only different species, they are animal and vegetable—in different kingdoms.

Previously, only one other case of trans-kingdom sex had been known: The crown gall tumor organism, a bacterium, is known to transfer genetic material to the injured root cells of certain higher plants. This phenomenon, while well known, had been thought unique.

"Now that this can be done with yeast as well, it opens the possibility that bacteria can transfer to other higher cells," said Patricia C. Zambryski of the University of California at Berkeley and co-author of a commentary on the research in the same issue.

Among the implications: Bacteria infecting animals might be able to transmit DNA to animal cells, a possibility that could explain some disease symptoms.

Trans-kingdom sex might also have evolutionary significance, providing a means of passing genetic information from one species to another.

At the moment, however, scientists do not know whether the transfer recorded by Heinemann and Sprague actually occurs in nature since the conjugation of bacteria and yeast was observed only by inserting special genetic markers in the bacteria.

"We don't know whether this happens in nature because the bacteria doesn't give us anything to detect," Zambryski said. "We don't know what to look for."

—William Booth, Malcolm Gladwell and Kathy Sawyer

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Conjugational Transfer of Antibiotic Resistance

Ability level ..... Grade 10 general biology – Grade 12 AP biology
Class periods required ..... 3 20-minute segments of 3 separate periods

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Introduction

Bacterial conjugation occurs frequently under natural conditions. It is the means by which pathogenic bacteria may acquire resistance to several different antibiotics. It is also a tool used in constructing rDNA vectors for genetic engineering of bacteria.

In this activity, an *E. coli* cell containing the F plasmid (Curtis & Barnes 1989) is the donor cell (+). The donor cell builds a tube or pilus that connects it to the recipient cell (-) lacking the F plasmid. The genes allowing the (+) cell to construct the pilus are on the F plasmid. One of the two strands of plasmid DNA is replicated by the (+) cell and passed over to the (-) cell through cytoplasmic bridges. Upon synthesis of the complementary strand by the recipient cell, it becomes a (+) cell capable of making a pilus.

Students will grow the (+) and (-) strains of *E. coli*. They then demonstrate that conjugation occurred by growing the individual and conjugated strains on plates containing antibiotics. Sterile conditions should be maintained at all points during the procedure.

Objectives

At the end of this activity, students should be able to:

1. Describe the steps involved in bacterial conjugation.
2. Relate antibiotic-resistance in bacteria to the conjugation process.
3. State how conjugation could be used in genetic engineering.
4. Demonstrate a controlled experiment.
5. Demonstrate some basic bacteriological procedures.
6. Recognize and understand the following words: **conjugation, plasmid, genetic engineering, pilus, ampicillin, streptomycin, negative control, positive control.**

Materials

For entire class

- 37°C incubator (if available)

Supplies (per lab team)

- propane, Bunsen or alcohol burners
- 1 test tube containing a 5 ml suspension of *E. coli* Strain C-I in Luria broth (LB) (This can be used by several different teams.)
- 1 test tube containing a 5 ml suspension of *E. coli* Strain C-II in LB (This can be used by several different teams.)
- 3 Luria broth (LB) agar plates without antibiotic
- 1 LB agar plate containing ampicillin
- 1 LB agar plate containing streptomycin
- 1 LB agar place containing ampicillin + streptomycin
- 1 sterile pipet (graduated to 0.1 ml or 100 µl)
- 25-50 ml methanol in shallow dish
- 1 inoculating needle
- 1 bacterial spreader
- 1 marking pen
- Lysol water

Teacher Preparation

(will require at least one hour)

1. Obtain *E. coli* Strains C-I and C-II from Department of Biology, Marquette University, Milwaukee, WI 53233.
2. Prepare suspensions by inoculating C-I and C-II strains each into separate tubes containing 5 ml sterile LB and incubating either overnight at 37°C or several days at room temperature.
3. Obtain bacterial growth plates from a commercial supplier or prepare them as follows:
   - For LB agar plates: To a liter of water add 10 g tryptone, 5 g yeast extract, 10 g NaCl, 15 g agar and 3.5 ml of 1.0 N NaOH solution.
   - For LB agar + ampicillin plates: Add 10 ml of 1% ampicillin solution per liter of LB agar plate solution.
   - For LB agar + streptomycin plates: Add 10 ml of 1% streptomycin solution per liter of LB agar plate solution.
   - For LB agar + ampicillin + streptomycin plates: Add 10 ml of 1% ampicillin solution and 10 ml of 1% streptomycin solution per liter of LB agar solution.
4. Prepare "Lysol" water by adding 30 ml of Lysol to 4 liters of water.

Teaching Tips

1. Be sure to review safety with students before proceeding. A demonstration of safe procedures by the instructor before the students begin is recommended.
2. Incubations can be done either overnight in a 37°C incubator or for several days at room temperature.
3. Sterile cotton swabs such as Q-tips may be substituted for the sterile pipet and spreader (Thomson 1988).

Procedure

(See student pages for detailed protocol.)

1. Students read the Background Reading.
2. Students prepare culture plates and leave them to incubate.
3. Students add bacteria to the antibiotic-containing plates and incubate.
4. Students compile class results and answer Questions.

Adapted from Thomson, R.G. (1988). Recombinant DNA made easy: 1. "Jumping Genes." *The American Biology teacher*, 50(2), 101-106.

Conjugational Transfer of Antibiotic Resistance

Answers to Student Questions

1. *Growth in the form of streaks should occur only on those sectors that received bacteria containing the resistance gene(s) for the antibiotic(s) in the plate. A streak of bacteria should develop on Sectors II and I + II of the LB/AMP plate, on Sectors I and I + II of the LB/STR plate and (provided conjugation occurred on Plate I + II) on Sector I + II of the LB/AMP/STR plate. No growth should occur on the other sectors.*

2. *See table below.*

3. *A control is a data point for which the experimental factor [in this case, the mixing of Strains I and II] is not present. It is used to help determine whether the experimental factor produced a real effect. Also, when experimental results do not turn out as expected, it can often be used to help determine why.*

   • **LB/AMP plate:** *No growth in Sector I (negative control) confirms presence of AMP in the plate and the lack of ampicillin-resistance gene (amp^r) in strain C-I.*

   Growth in Sectors II and I + II (positive controls) confirms the presence of amp^r in Strain C-II

   • **LB/STR plate:** *No growth in Sector II (negative control) confirms the presence of STR in the plate and the lack of streptomycin-resistance gene (str^r) in Strain C-II.*

   Growth in Sectors I and I + II (positive controls) confirms the presence of str^r in Strain I.

   • **LB/AMP/STR plate:** *No growth in Sectors I and II (negative controls) confirms the presence of AMP and STR in the plate.*

4. *Answers will vary. For instance, a student could argue that cells would be more likely to contact one another if they are free to move in a liquid medium. But another could argue that a pilus would be more likely to extend toward and contact a recipient cell on a solid medium.*

5. *Genetic engineering usually involves getting a cell to express foreign genetic material. The gene(s) must first get into the recipient cell in order to be expressed. Conjugation offers one means of transferring genes from one cell to another.*

Resources

Curtis, H. & Barnes, N.S. (1989). *Biology*. New York: Worth Publishers. 1192 pp.

Thomson, R.G. (1988). Recombinant DNA made easy: I. "Jumping genes." *The American Biology Teacher, 50* (2), 101-106.

Plate	Sector		
	I	II	I + II
LB/AMP	-	+	+
LB/STR	+	-	+
LB/AMP/STR	-	-	+

Conjugational Transfer of Antibiotic Resistance

STUDENT PAGE 1

Background Reading

Bacterial conjugation occurs frequently under natural conditions. It is the means by which pathogenic bacteria may acquire resistance to several different antibiotics. It is also a tool used in constructing rDNA vectors for genetic engineering of bacteria.

In this activity, an *E. coli* cell containing the F plasmid (Curtis & Barnes 1989) is the donor cell (+). The donor cell builds a tube or pilus that connects it to the recipient cell (-) lacking the F plasmid. The genes allowing the (+) cell to construct the pilus are on the F plasmid. One of the two strands of plasmid DNA is replicated by the (+) cell and passed over to the (-) cell through cytoplasmic bridges. Upon synthesis of the strand by the recipient cell, it becomes a (+) cell capable of making a pilus.

You will grow the (+) and (-) strains of *E. coli*. You will then demonstrate that conjugation occurred by growing the individual and conjugated strains on plates containing antibiotics. The donor (+) strain C-II contains a conjugative plasmid that carries a resistance gene to the antibiotic ampicillin. The recipient (-) strain C-I carries a chromosomal gene for streptomycin resistance. If conjugation occurs, C-I cells receive the plasmid from C-II cells, and grow on agar plates containing both ampicillin and streptomycin.

A variety of different methods have been employed to facilitate plasmid transfer between cells in the laboratory. These include methods using surfaces such as plates or membranes, as well as those involving transfer in nutrient broth. Some plasmids transfer with higher frequency with one technique, some with another. In this activity a plate transfer technique will be used. Sterile conditions should be maintained at all points during the procedure.

Conjugational Transfer of Antibiotic Resistance

STUDENT PAGE 2

Activity Sheet

Materials

For entire class

- 37°C incubator (if available)

Supplies (per lab team)

- propane, Bunsen or alcohol burners
- 1 test tube containing a 5 ml suspension of *E. coli* Strain C-I in Luria broth (LB) (This can be used by several different teams.)
- 1 test tube containing a 5 ml suspension of *E. coli* Strain C-II in LB (This can be used by several different teams.)
- 3 Luria broth (LB) agar plates without antibiotic
- 1 LB agar plate containing ampicillin
- 1 LB agar plate containing streptomycin
- 1 LB agar place containing ampicillin + streptomycin
- 1 sterile pipet (graduated to 0.1 ml or 100 µl)
- 25-50 ml methanol in shallow dish
- 1 inoculating needle
- 1 bacterial spreader
- 1 marking pen
- Lysol water

Procedure

Part I

1. Wipe down laboratory table with Lysol water.
2. Label three Luria broth (LB) plates as follows: "I," "II" and "I+II."
3. Use sterile pipet to transfer approximately 0.1 ml of Strain C-I suspension to Plate I.
4. Sterilize spreader by dipping in alcohol, then touching briefly to flame and allowing alcohol to burn off away from flame.
5. Cool spreader by rubbing on agar away from cell suspension.
6. Spread Strain C-I cells on plate with cooled spreader.
7. Resterilize spreader as in Step 4.
8. Repeat Steps 3-7 using C-I suspension and Plate I+II.
9. Repeat Steps 3-7 using C-II suspension and Plate II.
10. Repeat Steps 3-7 using C-II suspension and Plate I+II.
11. Invert plates and incubate.
12. Wipe down laboratory table with Lysol water.

Part II

1. Wipe down laboratory table with Lysol water.
2. Use marking pen to divide lower portion of LB agar + ampicillin plate into thirds. (Make sure plate is labeled as one containing ampicillin.) Mark sectors of plate "I," "II" and "I+II."
3. Divide the other two antibiotic-containing plates into thirds and mark the sectors as in Step 2 above.
4. Use inoculating needle to streak bacteria from Plate I on Sector I of each of the three antibiotic-containing plates.
5. Similarly streak bacteria from Plate II on Sector II and bacteria from Plate I+II on Sector I+II on each of the antibiotic-containing plates.
6. Invert antibiotic-containing plates and incubate.
7. Wipe down laboratory table with Lysol water.
8. Compile class results.

Conjugational Transfer of Antibiotic Resistance

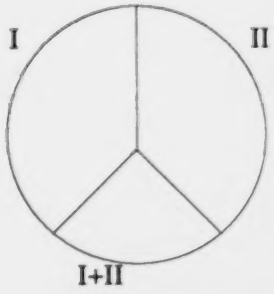
STUDENT PAGE 3

Questions

1. On what sectors do you expect to find bacterial growth, and why?

Plates LB/AMP

LB/STR



LB/AMP/STR

2. Examine your plates for bacterial growth. Fill in the table below. Use a "+" to indicate bacterial growth and a "–" to indicate no bacterial growth. Results from all lab teams will be pooled into a table of class results.

Plate	Sector		
	I	II	I + II
LB/AMP			
LB/STR			
LB/AMP/STR			

3. Of the nine sectors plated in this activity, eight are controls. What is a control?

What information can be obtained from the control plates in this activity?

- LB/AMP plate:

- LB/STR plate:

- LB/AMP/STR plate:

Conjugational Transfer of Antibiotic Resistance

STUDENT PAGE 4

4. You could design experiments to improve the efficiency of transfer from C-II to C-I in this procedure. For instance, the two strains might be cultured together in a tube containing liquid LB rather than on a plate. Do you think this would increase or decrease the number of cells that successfully conjugated? Justify your answer.

5. Explain how conjugation could be used in genetic engineering.

The Ti Plasmid

Ability level ..... Grade 9 biology - Grade 12 AP biology
Class periods required ..... 1, followed by 5-10 minute observations over several weeks

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Introduction

Crown gall disease affects a wide range of dicotyledonous plants, including grapes, stone fruits and ornamentals (Chilton,1983). The cause of this disease is *Agrobacterium tumefaciens*, a common soil bacterium. *A. tumefaciens* incorporates some of the genes from its Ti (tumor inducing) plasmid into the plant genome, causing the plant to form tumors (galls). Researchers have developed a method to immobilize these tumor-causing genes while maintaining the gene transfer abilities of *A. tumefaciens*.

The Ti plasmids of *A. tumefaciens* are useful vectors, or "gene taxis," in plant genetic engineering. First, the plasmid's tumor-causing opine synthesis and hormone genes are removed, "disarming" the plasmid. Researchers then isolate and incorporate the desirable genetic material into a "disarmed" *A. tumefaciens* plasmid. The bacterium and its engineered plasmids are inserted into a recipient plant, and the new genetic material is incorporated into the recipient cell's chromosome.. This technique has been used successfully on a number of dicots. Monocots are resistant to infection, however, so other vectors must be used to transform them.

This activity demonstrates a tool widely used by biologists in their attempts to improve commercially valuable crop plants. Students infect a plant with a bacterium and design their own experiments regarding the development of crown gall disease. It also provides a simple model of tumor development and growth for a unit on human cancer.

Ann Matthysse, University of North Carolina at Chapel Hill, and Mary Louise Bellamy, National Association of Biology Teachers, provided the procedure for infecting *Kalanchoe* with *Agrobacterium tumefaciens*.

Objectives

At the end of this activity students should be able to:

1. Describe the basic steps involved in creating a "genetically engineered" organism.
2. Explain how the formation of a tumor cell (phenotype) is related to changes in its genetic makeup (genotype).
3. Design a controlled experiment.
4. Demonstrate some basic bacteriological procedures.
5. Recognize and understand the following words: **crown gall disease, tumor, gall, *Agrobacterium tumefaciens*, plasmid, opines, cytokinins, auxins.**

Materials

For entire class

- propane, Bunsen or alcohol burners

For each lab team

- test tube rack
- 25-50 ml of methanol in shallow dish*
- 2 squares of Parafilm,1 cm X 1 cm
- 1 small test tube containing 5-10 ml of sterile water
- 2 small *Kalanchoe* or other potted dicotyledonous plants
- 1 sterile scalpel, razor blade or lancet*
- 1 permanent felt-tip pen
- 1 alcohol swab
- 1 agar slant of *A. tumefaciens*
- Lysol water
- 1 inoculating needle

* The alcohol is necessary only if the blade is reused during the procedure. It is unnecessary if two sterile blades (e.g. sterile blood lancets) are available per team.

Teacher Preparation

(will require at least 45 minutes)

1. *A. tumefaciens* can be ordered from biological supply houses.
2. *Kalanchoe* can be ordered or purchased from a local florist.
3. Prepare a short discussion of Procedure on student page 1.
4. Lysol water – add 30 ml (2 tablespoons) of Lysol to 4 liters (a gallon) of water.
5. Equipment and water can be sterilized with an autoclave or pressure cooker (15 lbs. for 15 minutes).

Teaching Tips

1. Be sure to review safety procedures with your students before proceeding, particularly if they will be working with an open flame and alcohol. **The entire procedure should be demonstrated by the instructor before students begin.**
2. Show a plant infected with a crown gall to students if one is available.
3. Different species of dicot plants can be tested for susceptibility. Plants in the mustard family (e.g. *Brassica*, broccoli, cauliflower) appear to resist infection by *A. tumefaciens*.

Procedure

(See student pages for detailed protocol.)

1. Students do the Background Reading.
2. Students follow the procedure to inoculate their plants with the bacteria.
3. Students answer the Questions.
4. Students keep track of observations for up to 12 weeks afterwards.

Answers to Student Questions

1. *Plant part infected (e.g. stem vs.leaf); position of leaf on plant; position on leaf (e.g. upper vs. lower surface, closer to vs. farther from petiole); age*

The Ti Plasmid

of plant; whether the plant has been infected previously

2. **A.** *The problem statement should be specific, accurate and "doable" within a given amount of time and resources.*

   B. • Book: *Use the card catalog*
   • Newspaper: *Use Newsbank*
   • Periodical (journal): *Use the Reader's Guide to Periodical Literature, Biological Abstracts*

   C. *A hypothesis is an idea or statement to explain an observation or theory; hypotheses can be directly or indirectly tested with a controlled experiment*

   D. • Control treatment – *the "normal" condition or treatment to which you compare the experimental treatment.*
   • Experimental treatment – *the control treatment plus some change in a variable*
   • Variable tested – *the "thing" that is different between the control and experimental treatments.*

   E. *Students should design a table, list or graph, etc. that includes heading and date. Graph axes should be clearly labelled, including units.*

   F. *The conclusion is a statement about the data; does it support accepting or rejecting the hypothesis?*

Resources

Printed material

Chilton, M.-D. (1983). A vector for introducing new genes into plants. *Scientific American, 48*(6), 50-59.

Drexler, et al. (1989). Natural genetic engineering. In *Advances in Genetic Technology* (pp. T1-T7).

Biological Sciences Curriculum Study. Lexington, MA: D.C. Heath and Co.

The Ti Plasmid

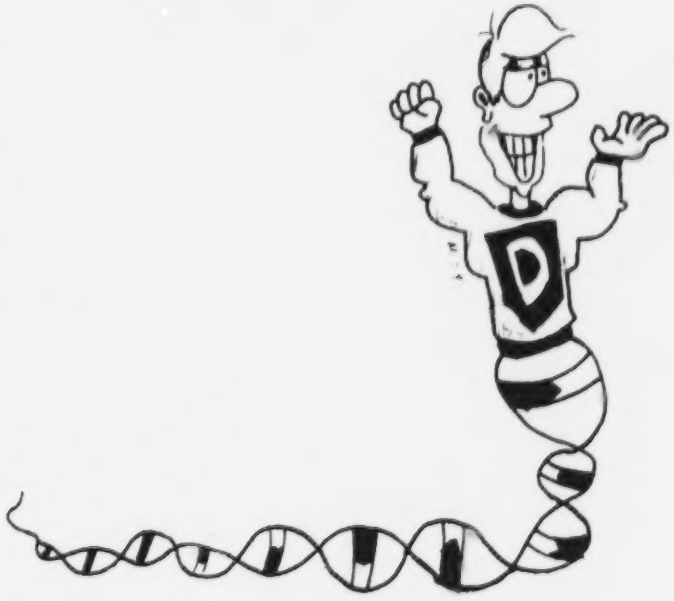
STUDENT PAGE 1

Background Reading

Agrobacterium tumefaciens, a common soil bacterium, causes a condition called "crown gall disease" in susceptible plants. Wounded plants release chemicals that attract *A. tumefaciens* to the wound site. These bacteria can attach to a plant cell and inject a portion of their DNA into the plant's cells. This DNA is then incorporated into the cell's chromosomes. The cell's metabolism is directed to make chemicals that are of no use to the plant, but provide a source of nutrition to the invading bacterium.

The injected DNA, called T-DNA, is part of a fairly large plasmid contained within the bacterial cell. Plasmids are circular pieces of DNA that contain only a few genes, none of which are essential to the bacteria's survival. The tumor-inducing (Ti) plasmid contains virulence (vir) genes that control the injection of T-DNA into the plant cell and its insertion into the plant cell chromosome. Some T-DNA genes allow the bacterium to use chemicals (called opines) produced by the plant cell. Other T-DNA genes produce the plant hormones cytokinins and auxins. The relative proportion of hormones produced determines the morphology of the gall or tumor; a predominance of cytokinins produces a shootlike ("shooty") tumor, and a predominance of auxins produces a rootlike ("rooty") tumor (Chilton 1983).

A. tumefaciens has turned out to be a very useful vector in the genetic engineering of certain plants. The challenge lay in figuring out how to "disarm" the tumor-forming genes in the Ti plasmid. After locating the genetic material to be inserted, researchers incorporated it into a "disarmed" *A. tumefaciens* plasmid and used the bacterium to introduce the new genetic material into a recipient plant cell. The disarmed plasmid, with the opine synthesis and hormone genes removed, did not cause the growth of a tumor, but did allow new genetic material to be incorporated into the recipient cell's chromosome. This technique has been used successfully on a number of dicotyledonous plants. Monocots are resistant to infection, however, so other vectors must be used to transform them.



The Ti Plasmid

STUDENT PAGE 2

Activity Sheet

Materials (To obtain from your teacher)

For each lab team

- 25-50 ml of methanol in shallow dish*
- 2 squares of *Parafilm*, 1 cm X 1 cm
- 1 small test tube containing 5-10 ml of sterile water
- 2 small *Kalanchoe* or other potted plants
- 1 sterile scalpel, razor blade or lancet*
- 1 permanent felt-tip pen
- 1 alcohol swab
- 1 agar slant of *A. tumefaciens*
- Lysol water
- 1 inoculating needle

* The alcohol is necessary only if the blade is reused during the procedure. It is unnecessary if two sterile blades (e.g. sterile blood lancets) are available per team.

Procedure

1. Wipe down the laboratory table with Lysol water.
2. Use an alcohol swab to wipe the plant surface to be wounded (stem or upper leaf).
3. Use a sterile scalpel to make a small, shallow wound on the plant surface. The wound should be deep enough to produce plant "juice," but should not go all the way through the stem or leaf. If a blade is to be reused, sterilize by dipping it in alcohol and touching it briefly to a flame.
4. Remove the cap from the test tube of water and flame the tube's mouth. Use a sterile inoculating needle to apply a small amount of sterile water to the wound. Flame the needle and reflame the mouth of the test tube before replacing its cap.
5. Remove the cap from the slant tube of *A. tumefaciens* and flame the tube's mouth. Flame an inoculating needle, then cool the needle. Use the sterile needle to apply a small amount of *A. tumefaciens* from the slant culture to the plant's wound. Reflame the needle. Reflame the mouth of the test tube before replacing its cap.
6. Remove paper from the sterile side of *Parafilm*. Apply *Parafilm* to the plant's wound. It does not have to adhere tightly to the plant surface, but it should remain in place for a day or so.
7. Repeat these same steps with a second plant **except** do not apply *A. tumefaciens*.
8. Wipe down all laboratory surfaces with Lysol water.
9. Between labs, equipment and water can be sterilized with an autoclave or a pressure cooker.
10. Water plants as you normally would. Evidence of gall formation may appear in 1-2 weeks; if this does not occur, however, do not discard the plants. Gall formation sometimes requires several months. Record your observations in the table below.

Date	Observations
Week 1	
Week 2	
Week 3	
Week 4	
Week 5	
Week 6	
Week 7	
Week 8	
Week 9	
Week 10	
Week 11	
Week 12	

The Ti Plasmid

STUDENT PAGE 3

Questions

1. After discussing experimental design with your teacher, list five other experimental factors you might test using these same plants and bacteria:

   a.
   b.
   c.
   d.
   e.

2. Use the following outline to describe your experiment:

   A. Define the problem (list three characteristics of a well-defined problem).

   B. Literature review (explain how you would look up the following materials at the library):

   - **Book :**
   - **Newspaper :**
   - **Periodical (journal) :**

   C. Hypothesis (define what a hypothesis is).

   D. Experimental design

   - **Control treatment:**
   - **Experimental treatment:**
   - **Variable tested:**

   E. Data collection (design a data collection sheet).

   F. Conclusion (explain how the conclusion relates to the hypothesis).

Jumping Genes in Corn

Ability level .....General, advanced biology, Grades 10 – 12
Class periods required .....2 – 3

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Introduction

For the first part of this century, geneticists believed that genes were static with regard to their loci or positions on chromosomes. However, in 1947 Barbara McClintock proposed that genes could indeed move within and between chromosomes. McClintock kept careful records of genetic crosses in corn. From her data, she concluded that there must be genetic elements that could move to and be inserted at the loci for color in corn kernels. These fragments of DNA are now called transposons. Although her work initially did not have much impact on genetic theory, it was later corroborated by findings of similar phenomena in bacteria and mammalian retroviruses. She received the Nobel prize for her work in 1983.

In order to get a gene from one kind of cell to be expressed in a different kind of cell, frequently the gene must be inserted in the recipient cell's chromosome. Transposons are a useful tool for genetic engineering in plants. Like certain viruses, they provide a mechanism for inserting foreign DNA into a host cell's chromosome. They have been used in research laboratories in the sometime complex series of steps required to obtain a plant that expresses a gene from another organism.

This lesson is composed of three activities, each building on or extending what was learned in the previous one. Activity I involves simple Mendelian inheritance, Activity II deals with the complex genetic mechanisms involving gene interaction and Activity III introduces the concept of how transposons (jumping genes) can affect the color of "Indian" corn kernels. Parts I and III are suitable for general level students. The entire lesson is suitable for college prep, accelerated and advanced students.

Objectives

At the end of this activity students should be able to:

1. Use Mendelian principles to explain certain aspects of the determination of kernel color and morphology in corn (Activity I).
2. Use non-Mendelian principles to explain further the determination of kernel color (Activity II).
3. Define transposon and describe how transposons (jumping genes) can affect kernel color and morphology (Activity III).
4. Explain how transposons are used to insert foreign genetic material into plants (Activity III).
5. Appreciate that there are various levels at which a scientific problem can be understood.
6. Appreciate that the hereditary material of a cell is a dynamic, constantly changing system, of which gene movements and interactions are an important part.
7. Recognize and understand the following words:
   Activity I: embryo, gametes, alleles, Mendelian genetics, pericarp, aleurone layer, endosperm, Punnett square
   Activity II: epistasis, locus, multiple alleles,
   Activity III: transposon, dissociation, autonomous, translocation

Materials

per lab team

▼Ears of the following commercially available genetic corn (expressed as a ratio of kernels). At least one ear is needed per group:

Activity I:

▼ 1 yellow starchy: 1 yellow sweet
▼ 3 purple starchy: 1 yellow starchy
▼ 9 purple starchy: 3 purple sweet: 3 yellow starchy: 1 yellow sweet.

Activity II:

▼ 9 purple: 3 red: 4 white
▼ 12 purple: 3 yellow: 1 white
▼ 13 yellow: 3 purple
▼ 2 purple: 1 yellow: 1 white
▼ 9 red: 7 white
▼ 9 purple: 3 red: 3 yellow: 1 white
▼ 3 yellow: 1 purple
▼ 9 yellow: 3 white: 4 purple

Activity III:

▼ "Indian corn"

Teacher Preparation

Order the corn.

Teaching Tips

These three activities should be taught several days apart rather than on consecutive days to allow students to assimilate the information. This strategy will also provide a unifying theme for other components of a genetics unit.

Before Activity I:

1. Review simple dominance, Mendel's laws and Mendelian ratios with the students.

Appreciation is expressed to Tom Peterson of Cold Spring Harbor for help with technical aspects of this activity.

Jumping Genes in Corn

2. Briefly describe the status and basic structure of corn kernels using information from the student pages.
3. Use the table at right for the class discussion.

	R/r	Su/Su
purple/starchy	R_	Su_
purple/sweet	R_	susu
yellow/starchy	rr	Su_
yellow/sweet	rr	susu

Before Activity II:

1. Review the results of Activity I.
2. Describe the more complex mechanisms underlying the inheritance of kernel morphology using information from the student pages.

	Pr/pr	R/r	C/C^I/c	Y/y
Purple	Pr_	R_	CC or Cc	__
Red	prpr	R_	CC or Cc	__
Yellow	__	__	C^I_ or cc	Y_
Yellow	__	rr	__	Y_
White	__	__	C^I_ or cc	yy
White	__	rr	__	yy

Before Activity III:

1. Review the results of Activity II.
2. Describe transposons in corn and how they may affect kernel color using information from the student pages.
3. It's recommended that students begin each of these three activities by working in groups, then work individually to ensure that each understands the concepts involved. (The number of ears available may dictate that students work in groups).

Procedure

(See student pages for detailed protocol.)

1. Students do Background Reading.
2. Students work through the steps of Activity I and present their conclusions to the class.
3. Students work through the steps of Activity II and present their conclusions to the class.
4. Students work through the steps of Activity III and present their conclusions to the class.
5. Students answer the Questions.

Answers to Student Questions

Activity II Procedure

Some other possibilities include:

- *Prpr Rr YY CC X Prpr Rr Yy cc*
- *Prpr Rr yy CC X Prpr Rr YY Cc*
- *Prpr RR YY Cc X Prpr RR yy Cc*

Questions

1. *Its endosperm would be yellow, but the color would be masked by the purple in the overlying aleurone. The kernel would appear purple.*
2. *1 purple: 1 red: 1 yellow: 1 white*
3. *PrPr Rr yy Cc X __ Rr yy Cc*
4. *Answers will vary depending in part on the students' background in genetics. Some might suggest that the C^I gene codes for a protein that destroys purple and red pigment in aleurone cells. Others might suggest that the C^I gene product somehow prevents the Pr and R gene products from being produced, perhaps by binding to the chromosome and preventing transcription. Still others might suggest that the C gene codes for a product that is necessary for the production of purple and red pigment, and that the C^I product prevents the C product from being made. There are also other possibilities.*
5. *Genetic engineering usually involves getting a cell to express foreign genetic material. Appropriate expression can depend on inserting the genetic material at a specific chromosome locus. Transposons provide a means for controlling with precision where it is inserted.*

Resources

Audio-visuals

"The Biological Revolution: 100 Years of Science at Cold Spring Harbor" contains a short piece on McClintock's work. It is published by Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724. Also available from: Cabisco Biotechnology, Burlington, NC 27215; 800-334-5551 or 800-632-1231 (NC only).

Printed materials

Federoff, N.V. (1984). Transposable genetic elements in maize. *Scientific American, 250*(6), 85-98.

Sprague, G. & Dudley, J.W. (1988). *Corn and corn improvement* (3rd ed.). Madison, WI: American Society of Agronomy.

Computer software

Have your students design and write a computer program that simulates the mechanisms of inheritance in corn. Such a program could be modeled after the CATLAB software developed by Judith Kinnear and published by Conduit, The University of Iowa, Oakdale Campus, Iowa City, Iowa 52242.

Jumping Genes in Corn

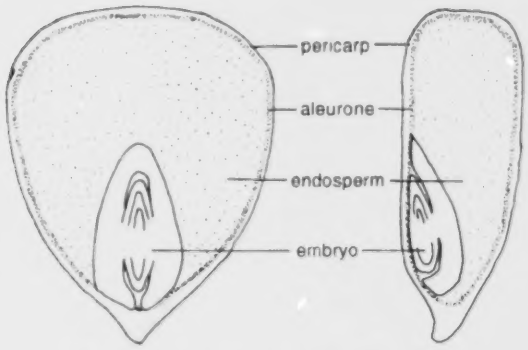
STUDENT PAGE 1

Background Reading

Activity I:
Simple Inheritance

Each kernel on an ear of corn is a potential corn plant. It contains an embryo resulting from the union of two gametes, one (the egg) contributed by the female plant part and the other (the sperm) contributed by the male plant part.

The structure of a corn kernel is illustrated below. It includes a thin outer layer, the pericarp; a thin underlying aleurone, which is the outermost layer of the endosperm; the remainder the endosperm, which contains the kernel's stored food; and the embryo. Each of the three outer layers may be pigmented or not, with the presence or absence of pigmentation being under the control of several different genes.



Drawing adapted from Fedoroff, N.V. (1984). *Scientific American, 250*(6), 85-98.

The chemical form of food stored in the endosperm affects the appearance of the kernel. This is under genetic control, with the Su gene for starchy (smooth and rounded appearance) being dominant to the su gene for sweet (wrinkled and raisin-like).

Each of the three outer layers may be pigmented or not. Pigmentation in the aleurone layer is under control of a number of genes. In Activity I, you will be concerned with only one of these genes, the R locus. R is dominant to r and causes a purple or red color; the r allele does not cause any pigmentation of the aleurone.

Activity II:
Complex Inheritance

Pigmentation in the aleurone layer is actually under the control of at least three different genes, R, Pr and C, each of which may occur in different forms, or alleles. At the R locus, allele R allows color to be present in the aleurone; R is dominant to r which does not produce a pigment. The expression of a second set of alleles at a different locus, the Pr locus, varies with whether the R or r allele is present. Pr produces purple color, while pr contributes red. Pr is dominant to pr. In order for the Pr locus to be expressed, there must be at least one R allele present. If there are at least one R gene and one Pr gene (i.e., a Pr_ R_ genotype) then the kernel can be purple; a genotype of prpr R_ can produce a red aleurone; _ _ rr contributes no color to the aleurone. The relationship between the R and Pr loci is an example of epistasis, a condition that results when genes at one locus affect the expression of genes at another locus.

Note that we said the aleurone **can** be purple. Aleurone color is affected by yet another locus, C, which provides an example of both epistasis and multiple alleles. In order for the aleurone to be pigmented, there must be at least one C allele at the C locus, i.e., CC or Cc. A cc individual will lack aleurone pigmentation. Besides C and c, there is another allele C^1; it is dominant to C and prevents any color from being expressed in the aleurone. For instance, a PrPr RR C^1C individual would lack aleurone coloration. A locus with more than two alleles is said to be under the control of multiple alleles.

Another locus that is involved in determining the color of corn kernels is the Y locus. This is a case of simple dominance, with Y_ producing yellow endosperm and yy producing white endosperm. The color of the endosperm is often not visible because of the overlying purple or red pigments in the aleurone.

Jumping Genes in Corn

STUDENT PAGE 2

Activity III: Jumping Genes and Inheritance

For the first part of this century geneticists believed that genes were static with regard to their positions on chromosomes. Work first reported by Barbara McClintock in 1947, however, revealed that genes could indeed move within and between chromosomes. McClintock kept careful records of genetic crosses in corn and observed the color changes on individual kernels of ears of corn over numbers of generations. From her data, she concluded that there must be genetic elements that could move to and be inserted at the loci for color in corn kernels. These fragments of DNA are now called transposons. or transposable genetic elements. When she published her unorthodox work in the early 1950s, it was discounted as impossible because it did not support the hypothesis under which all other geneticists were operating, that is, that the position of genes was fixed. Her work was later corroborated by findings of similar phenomena in bacteria, yeast, fruit flies and mammalian retroviruses. She received the Nobel prize for her work in 1983.

Specifically, McClintock showed that there was a genetic element which could move to and be inserted at the C locus. Insertion of this Ds element (for dissociation, so named because it was frequently a site of chromosome breakage) rendered the C locus unable to contribute to the production of purple pigment in the aleurone; subsequent removal of the Ds element from the C locus allowed the pigment to be produced. Ds did not itself cause the breakage, however; rather this required the presence of another element, called Ac for Activator. If the Ds element (in the presence of the Ac element) moved from the C locus during the development of a kernel, all cells resulting from that cell would be purple. Each cell in which such a translocation took place could eventually result in a purple spot on an otherwise yellow or white kernel of corn.

The P locus was another found to affect kernel color. It was found that the Ac element could insert itself at the P locus, disrupting thereby the production of a red-orange pigment in the pericarp. Translocation to and from the locus several times during kernel development resulted in the red-orange swirls characteristic of many kernels on "Indian corn."

Subsequent studies in corn have revealed other "families" of transposons analogous to the Ac-Ds family. Each family contains elements that, like Ac, can effect their own movement and that of other elements in the family from one site to another. Such elements are called autonomous. In addition, each family generally contains nonautomonous elements that, like Ds, can only move in the presence of an autonomous member of the same family. In many cases nonautonomous elements appear to be derived from autonomous elements by deletion of DNA sequences.

Jumping Genes in Corn

STUDENT PAGE 3

Materials

Each lab group will need one of the following ears of corn

Activity I:

- 1 yellow starchy: 1 yellow sweet
- 3 purple starchy: 1 yellow starchy
- 9 purple starchy: 3 purple sweet: 3 yellow starchy: 1 yellow sweet

Activity II:

- 9 purple: 3 red: 4 white
- 12 purple: 3 yellow: 1 white
- 13 yellow: 3 purple
- 2 purple: 1 yellow: 1 white
- 9 red: 7 white
- 9 purple: 3 red: 3 yellow: 1 white
- 3 yellow: 1 purple
- 9 yellow: 3 white: 4 purple

Activity III:

- "Indian corn"

Procedure

Activity I:

Simple Inheritance

1. Obtain an ear of corn for your group.
2. Using slides or the ears themselves, point out examples of purple vs. yellow, and starchy vs. sweet kernels.
3. Purple color is caused by pigmented cells in the aleurone. It is under control of a pair of alleles, R which allows the color to be expressed and r which does not allow expression. R is dominant tor r. Smooth, round kernels (called "starchy") result from the presence of a dominant gene Su, whereas wrinkled kernels (called "sweet") have the genotype susu.
4. Determine the genotypes of the parents that produced your ear of corn. **Hint:** Count the number of kernels of each phenotype, then make a Punnett square and "reason backward" to obtain the parents' genotypes. In the interest of time you can count only a few rows rather than the whole ear.
5. Choose a spokesperson (if working in a group) to present your results in a whole class discussion.

Activity II:

Complex Inheritance

1. Obtain an ear of corn for your group.
2. Make sure you can distinguish purple, red, yellow and white kernels
3. Use the information in the Background Reading to determine with your group the possible genotypes of purple, red, yellow and white kernels.
4. Determine the genotypes of the parents that produced your ear of corn. Two hints that may be of use to you are:

Hint 1: Look for examples of familiar Mendelian ratios. For instance, an ear that has 9 purple: 3 red: 4 yellow has a non-yellow:yellow ratio of 3:1. Such a ratio could have resulted from crossing two heterozygous parents Rr X Rr. In addition, the ratio among the non-yellow kernels is 3 purple: 1 red, suggesting parental genotypes of Prpr Rr X Prpr Rr. The fact that all the kernels lacking aleurone pigment are yellow suggests that at least one parent was homozygous for the Y gene, i.e. Prpr Rr YY X Prpr Rr __. These parental genotypes account for the 9:3:4 ratio provided at least one parent was homozygous for C and neither had C^1, giving one possible answer of Prpr Rr YY CC X Prpr Rr yy CC. What are some other "correct" answers?

Hint 2: As an alternative, it may be helpful to make a Punnett square and "reason backward" to obtain the parents' genotypes.

5. Choose a spokesperson (if working in a group) to present your results in a whole class discussion.

Activity III:

Jumping Genes and Inheritance

1. Obtain an ear of "Indian corn" for your group.
2. Ask each group to look for examples of:
   - kernels with purple or white spots.
   - kernels with red-orange swirls resulting from translocations to/from the P locus.
   - Other color patterns that might be explained by similar genetic mechanisms.
3. Use the information in the Background Reading to determine how:
   - purple or white spots result from translocations to/from the C locus.
   - red-orange swirls result from translocations to/from the P locus.
4. Choose a spokesperson (if working in a group) to present your results in a whole class discussion.

Jumping Genes in Corn

STUDENT PAGE 4

Questions

1. Assume a corn kernel had the genotype Prpr Rr YY Cc. What color would its endosperm be? What color would the kernel appear to be? Explain your answers.

2. Assume that an ear of corn is produced by the following cross:

   Prpr RR Yy CC X prpr RR yy CC^1

   What would the phenotypic ratio of the kernels on the ear be?

3. Assume that the phenotypic ratio on an ear of corn is 9 purple: 7 white. What were the genotypes of the parents?

4. How do you think the C^1 gene prevents pigment from occurring in the aleurone even when the Pr, R and C genes are present?

5. How might transposons be used one day in genetic engineering?

DNA Fingerprinting

Ability level ..... Biology 1, AP biology
Class periods required ..... Activity A: 1; Activity B: 2 + 1 homework assignment; Activity C: 2 + 1 homework assignment

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Introduction

(Note that this lesson requires EDVOTEK Kits # 102 and #109 and electrophoresis apparatus.)

There are three parts to this lesson:

Activity A: Students read about the function of restriction enzymes and then complete a written exercise clarifying the role of silent mutations in producing two different sized DNA bands in restriction digests.

Activity B: Based on Edvotek Kit 102 *"Analysis of Restriction Enzyme Cleavage Patterns of DNA."* Students read the Pre-lab information as homework. In class they cast a gel and subsequently run, develop and analyze the effects of two restriction enzymes (Bgl I and Eco RI) on three kinds of DNA (plasmid, lambda phage and bovine chromosomal DNA). This activity allows students to see the various sized bands produced by restriction enzymes and discuss possible reasons for their migration rates. Students then do a post-lab analysis worksheet.

Activity C: Based on Edvotek Kit 109 *"DNA Fingerprinting - Identification of DNA by Restriction Fragmentation Patterns."* Students read the pre-lab information as homework. In class, they cast a gel and subsequently run and analyze patterns produced by the digesting of three samples of (non-human) DNA from the crime scene, and two suspects with Eco RI and Sma I. They can then identify the suspect at the scene of the crime. A post-lab analysis worksheet is provided.

Objectives

After this activity students should be able to:

1. Describe the steps involved in the process of DNA fingerprinting.
2. Explain how restriction enzymes produce different-sized DNA bands on agarose gels.
3. Recognize similar patterns in the analysis of DNA fingerprints.
4. Cast and run agarose gels and then analyze DNA band patterns produced by restriction enzymes and DNA fingerprinting.
5. Discuss the need for establishing laboratory standards for DNA fingerprinting techniques.
6. Recognize and understand these words: **restriction enzymes, repetitive sequences, alleles, silent mutations/wobble, genetic code, protein synthesis, probe/radioactive probe, agarose gel electrophoresis, restriction fragment length polymorphisms.**

Materials

- Copies of pre-lab readings, lab instructions and post-lab analysis worksheets.
- **Edvotek Kit 102** (enough for two experiments)
  Kit includes: 8 tubes of different DNA fragments, 1 tube of practice gel loading solution, 4 plastic microtipped pipets, 2 grams of agarose, 1 bottle electrophoresis buffer, 2 bottles methylene blue staining reagents.
- **Edvotek Kit 109** (enough for two experiments)
  Kit includes: 6 tubes of different DNA samples , 1 tube of practice gel loading solution, 4 plastic microtipped pipets, 2 grams of agarose, 1 bottle of electrophoresis buffer, 2 bottles of methylene blue staining reagents.

Both kits require

- horizontal gel electrophoresis apparatus
- DC power supply
- gel scooper
- gel staining tray
- 1 gallon distilled water
- gloves and safety goggles

Teacher Preparation

1. Order kits from: EDVOTEK, Inc., P.O. Box 1232, West Bethesda, MD 20817; 800-Edvotek.
2. Make copies of EDVOTEK lab instructions for each lab team.
3. Make copies of pre-lab readings and post-lab worksheets for each student.
4. Follow the kit instructions ("Pre-Lab and Special Instructions") to prepare the gels (approximately 40 mins.).
   Note: You may wish to have your students prepare the gel, which will require a class period.

Teaching Tips

A. Review protein synthesis with the class before starting.
B. Gels for kits 102 and 109 can be run at the same time to reduce the amount of class time required.
C. Consult kit directions for information concerning preparation of practice gels and practice-loading solutions. With these inexpensive substitutes, all students could practice gel-loading techniques.
D. You may wish to have a student videotape gel preparation to show subsequent students.

Procedure

Activity A

1. Students read "What are Restriction Enzymes?"
2. Students complete "Silent Mutations" worksheet.

Activity B

1. Students read and do the Pre-lab exercise for Kit 102. (Homework)
2. Students follow the "Practice Gel Loading Instructions" in Kit 102 (gel has already been prepared by the teacher).
3. Students follow the "Experiment Procedure" included in the kit.
4. Students follow the staining directions on the "Visualization of Nucleic Acids" sheet included with the kit.
5. Students complete the Post-lab analysis worksheet for Kit 102.

DNA Fingerprinting

Activity C

1. Students read "What is DNA Fingerprinting?" (Homework)
2. Read and do the Pre-lab worksheet for Kit 109. (Homework)
3. Students follow the "Experiment Procedure" included in Kit 109 (they should not require a practice gel if they have already done Activity B).
4. Students follow the staining directions on the "Visualization of Nucleic Acids" sheet included with the kit.
5. Students complete the Post-lab analysis worksheet for Kit 109.

Answers to Student Questions

Pre-lab Exercise for Kit # 102

1. *To develop an understanding of the use of restriction endonucleases as tools to cut DNA at specific nucleotide sequences.*
2. A. *Plasmid DNA (uncut)*
   B. *Plasmid cut with Bgl I*
   C. *Plasmid cut with Eco RI*
   D. *Lambda DNA (uncut)*
   E. *Lambda DNA cut with Eco Ri*
   F. *Lambda DNA cut with Bgl I*
   G. *Chromosomal DNA (uncut)*
   H. *Chromosomal DNA partically cut with Eco RI*
3. *Endonucleases which catalyze the cleavage of the phosphate bonds within both strands of DNA.*
4. a. Define: *the specific double-stranded sequences of DNA bases a restriction enzyme requires before it can cut.*
   b. 1) Symmetrical: *Both DNA strands in the site have the same base sequences when read in the 5' to 3' direction.*
   2) Cuts are opposite each other: *Both DNA strands have "blunt" ends rather than staggered ends.*
   3) Degenerate: *The recognition sites contain variable base positions.*
5. *The longer the DNA molecule, the greater the possibility that a given recognition site will occur.*
6. a. *A powerful separation method which uses an agarose gel through which electric current is sent.*
   b. *Neutral pH.*
   c. *According to their size and shape.*
   d. * supercoiled DNA: *a compact and entangled twisting of the DNA molecule*
   * nicked DNA: *DNA in which a phosphate bond of one of the double strands is broken*
   * catenanes: *interlocking rings formed by plasmids during replication*
   * dimer, trimer: *two interlocking plasmid rings; three interlocking plasmid rings*
   e. *From fastest to slowest: supercoiled DNA, nicked DNA, dimer, trimer.*
7. a. Supercoiled plasmid DNA
   number of base pairs: *4500*
   number of restriction sites for Bgl I :*1* Eco RI :*2*
   b. Lambda
   number of base pairs: *49000*
   number of recognition sites for EcoRI: *5*
   c. Bovine chromosomal DNA
   number of base pairs: *over a billion*
   number of recognition sites for Eco RI: *500,000 approx.*
8. a. Gel concentration, % of agarose: *8%*
   b. Running time for M20 chamber: *from 40 min. to 3 hours*
   c. Stain: methylene blue - *30 mins (interacts with DNA)*
   d. Destain in distilled water - *2 to 24 hours*

Post-lab Analysis for Kit #102

Tube A

Contents: *Plasmid DNA (uncut)*
Number of bands: *Usually 3. Results may vary.*
Your interpretation: *If more than one band appears, then catananes may have formed. The fastest band (the one farthest from the well) would be the uncut plasmid.*

Tube B

Contents: *Plasmid cut with Bgl I*
Number of bands: *1*
Your interpretation: *Since the plasmid is circular and has only 1 recognition site for Bgl I, only one band appears.*

Tube C

Contents: *Plasmid cut with Eco R I.*
Number of bands: *1*
Your interpretation: *Since the plasmid is circular and has two recognition sites for Eco RI, two bands of unequal length appear.*

Tube D

Contents: *Lambda DNA uncut*
Number of bands: *1*
Your interpretation: *Lambda DNA is a much larger molecule than plasmid DNA and does not migrate very far in the gel.*

Tube E

Contents: *Lambda DNA cut with Bgl I*
Number of bands: *5 or 6*
Your interpretation: *Since lambda DNA is linear and has five recognition sites, there are six bands produced. Only five bands may appear. This could be due to two pieces of DNA of similar length which then appear as one band. Also one band may be so small it does not have enough DNA to be visible when stained with methlyene blue dye.*

Tube F

Contents: *Lambda DNA cut with Bgl I*
Number of bands: *Results will vary. May get several bands that appear as a "blur."*
Your interpretation: *Lambda DNA has 29 recognition sites for Bgl I. Many of the bands may be too small to appear when stained with methylene blue. Also bands of similar size may be superimposed and appear as one band. See also interpretation for tube E.*

Tube G

Contents: *Chromosomal DNA uncut*
Number of bands: *1*
Your interpretation: *Appears as one band which does not migrate very far from the well since it is such a large molecule. May appear as a smear due to the very large size. See also tube H.*

Tube H

Contents: *Chromosomal DNA partially cut with Eco RI*
Number of bands: *A smear*
Your interpretation: *Due to the very large size of this sample, it is randomly sheared to smaller fragments. In addition, it has approximately 500,000 recognition sites for Eco R I.*

Conclusions: *Conclusions will vary.*

DNA Fingerprinting

Pre-lab Worksheet for Kit #109

1. *To develop a basic understanding of DNA fingerprinting by analyzing variations in restriction enzyme cleavage patterns obtained from different DNA molecules. To identify the source of an unknown DNA sample using the logic of fingerprinting.*
2. A. *DNA sample from crime scene cut with Eco RI*
   B. *DNA sample from crime scene cut with Sma I*
   C. *DNA sample from Suspect 1 cut with Eco RI*
   D. *DNA sample from Suspect 1 cut with Sma I*
   E. *DNA sample from Suspect 2 cut with Eco RI*
   F. *DNA sample from Suspect 2 cut with Sma I*
3. *The electrophoretic analysis of DNA fragment sizes generated by restriction enzymes.*
4. a. DNA in the average human chromosome contains *100 million* base pairs.
   b. Eco RI might cut human DNA into *750, 000* fragments
5. *A large number of alleles; mutations; deletions*
6. *(See Activity A, the wobble effect.) A change in the third base of a codon may change a base in a recognition site.*
7. *They cause individual variations in the distances between recognition sites of restriction enzymes.*
8. *Restriction fragment length polymorphisms are variations in DNA sequences between individuals as determined by differences in restriction enzyme cleavage patterns.*
9. *skin, blood, semen, hair roots*
10. a. *A DNA fragment that contains base sequences which are complementary to the variable arrays of tandemly repeated sequences.*
    b. *The probe, when labeled with radioactive isotopes, can be used to hybridize to the fragment containing complementary repeated sequences. Discuss autoradiography if the class has the background to assimilate the information.*
11. *A crime has been committed. Two suspects were apprehended. Hair specimens different from the victim's were found at the crime scene. DNA from the hair roots was extracted and purified.*
12. Agarose gel concentration: *8%*
    Running time for an M 20 apparatus: *between 40 min. and 2 hours.*

Post-lab Analysis for Kit #109

1. Lane 1: *crime scene sample cut with Eco RI*
   Lane 2: *crime scene sample cut with Sma I*
   Lane 3: *suspect 1 sample cut with Eco RI*
   Lane 4: *suspect 1 sample cut with Sma I*
   Lane 5: *suspect 2 sample cut with Eco R I*
   Lane 6: *suspect 2 sample cut with Sma I*
2. *Suspect 2. The bands in lanes 5 and 6 matched the bands in lanes 1 and 2. These samples were cut with Eco RI and Sma I respectively. Sample from suspect 1 cut with Sma I did not match the DNA sample from the crime scene cut with Sma I.*
3. *No. When the DNA samples from suspect 1 and 2 were cut with Eco RI, the resulting bands were identical. So it was necessary to treat the DNA samples with another enzyme, Sma I, which did produce a different band pattern.*
4. *Answers will vary.*

Additional Activities

Questions for class discussion

1. What other uses can you devise for the paternity testing technique besides determining who is the child's father?

2. When DNA fingerprinting evidence is used in a jury trial, the jurors need to understand the concepts involved. What information would you present to a jury to prepare them for the understanding of DNA fingerprinting? The creation of visual props would be useful.

3. What chain-of-evidence procedures must be followed in collection and testing of DNA samples so that the evidence is admissible in a court of law?

Projects

Students can work individually or in teams:

1. Recently there has been much public discussion about the need for appropriate laboratory standards for DNA fingerprinting when used in court. What case(s) prompted this public discussion? What standards are needed to make this technique wholly reliable as evidence in criminal proceedings?
   Consult the following references (see list under Resources) as starting points: Lander, Lewin and Zurer, and "Misprints."
2. DNA fingerprinting is an application of RFLPs technology. RFLPs are also being used to diagnose genetic diseases such as cystic fibrosis, sickle cell anemia and Huntington's disease.
   a. Consult *Advances in Genetic Technology* Lesson 5 (see reference list), which offers several activities focusing on principles involved in the procedures for detecting some genetic diseases.
   b. Research RFLPs technology and/or some of the diseases which can be detected through this technique. White's *Scientific American* article would be a good starting point.

Resource Materials

Biological Sciences Curriculum Study. (1989). *Advances in genetic technology*. Lexington, MA: D.C. Heath and Company. Lesson 5, "What are RFLPs?" provides a current paper and pencil activity on this topic of restriction fragment length polymorphisms.

BIO-REEP (Biotechnology Resource Educational Partnership), National Association of Biology Teachers, EDVOTEK, Inc., Georgetown University and National Science Foundation. Contact EDVOTEK, Inc., P.O. Box 1232, West Bethesda, MD 20827; 800-EDVOTEK.

Kieffer, G.H. (1987). *Biotechnology, genetic engineering and society:* Monograph Series III. Reston, VA: National Association of Biology Teachers. Several chapters provide concise discussion of applications of biotechnology.

DNA Fingerprinting

Lander, E.S. (June 15). DNA fingerprinting on trial. *Nature*, 339, pp. 501-505. Describes and explains the need for standards in this rapidly expanding forensic technique. It would be useful in providing the details for classroom analysis and discussion on the topic.

Lewin, R. (1989). DNA typing on the witness stand. *Science*, *244*(4908), 1033-1035. Discusses the need for standards in DNA typing. A more general presentation of various viewpoints.

Lewin, R. (1989). Misprints, science and the citizen. *Scientific American*, *261*(2), 12-14.

Moody, M.D. (1989). DNA analysis in forensic science. *Bioscience*, 39(1), 31-36. Provides a complete discussion of DNA analysis techniques and is a valuable source of background information.

Neufeld, P.J. & Coleman, N. (1990). When science takes the witness stand. *Scientific American*, *262*(5), 46-53. Discusses the problems arising from the application of forensic technology to criminal investigations. Includes both a scientific and legal analysis of DNA fingerprinting.

White, R. & LaLouel, J.-M. (1988). Chromosome mapping with DNA markers. *Scientific American*, *258*(2), 40-48. Explains fully the principles underlying restriction fragment length polymorphisms analysis, RFLPs. Diagrams are particulary helpful.

Zurer, P. (1989). DNA profiling on trial: Judge bars unreliable results. *Chemical and Engineering News*, *67*(34), 6-7. Brief news update summarizing the reasons why the DNA tests on the blood stains found on Joseph Castro's watch were ruled as unreliable.

Cellmark Diagnostics publishes a pamphlet which provides further information about the uses of paternity testing: CELLMARK Diagnostics, 20271 Goldenrod Lane, Germantown, MD 20874; 800-USA LABS or 301-428-4980

DNA Fingerprinting

STUDENT PAGE 1

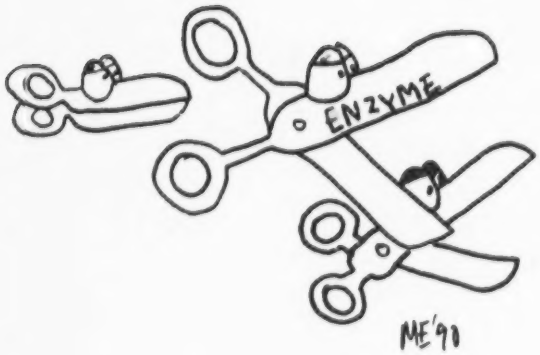
Activity A: Background Reading

What Is a Restriction Enzyme?

Restriction enzymes are endonucleases which catalyze the cutting or cleavage of both strands of DNA at the phosphodiester bonds. The restriction enzyme recognizes a specific sequence of DNA bases. The sizes of the resulting fragments, RFLPs, depend upon the distance between recognition sites which, in turn, depend upon several factors.

Twenty to thirty percent of human DNA is made up of highly repetitive DNA, much of it five to ten base pairs in length. For example, TTAGGG is found in the tips of human chromosomes and is repeated over and over: TTAGGG/TTAGGG/ TTAGGG/ etc. The length and quantity of repetitive sequences varies from individual to individual. If recognition sites border repetitive base sequences, then the sizes of the restriction fragments, (RFLPs), or "DNA fingerprints" produced by restriction enzyme digests will be different from one person to another (except for identical twins.)

Additionally, individuals in a population may possess different forms of the same gene, called "alleles." One allele may contain the sequence of a recognition site and the other allele may not. An individual may have the same amino acid sequence in a certain protein but have a "silent" mutation: a change in one of the three base pairs which code for a certain amino acid. The base pair at the 3' end of the mRNA codon can change but still attract the proper tRNA carrying the same amino acid. For example, AC codes for threonine. This phenomenon is called a wobble.



DNA Fingerprinting

STUDENT PAGE 2

Activity A: Silent Mutations Worksheet

Complete after reading "What Is a Restriction Enzyme?"

This activity illustrates how a silent mutation may change the recognition site of a restriction enzyme but not alter the phenotype: the amino acid sequence of a protein. Because the mutation does not affect the phenotype, it is appropriately called "silent." However this kind of mutation may affect the outcome of a DNA fingerprint. See Activity C: "What Is DNA Fingerprinting?"

The restriction enzyme Sma I, isolated from *Serratia marcescens* S_b, has the recognition sequence:

```
5' CCC↓GGG 3'
```

```
          Sma I
DNA 5' AAC CCG GGC 3'

mRNA 5' AAC CCG GGC 3'

        asn pro gly     (asparagine-proline-
                         glycine)
```

RNA polymerase copies the DNA strand from its complementary 3' to 5' strand. Now consider the following sequence:

```
DNA 5' AAT CCG GGC 3'

mRNA 5' AAU CCG GGC 3'
```

Due to the "wobble," the amino acid sequence is still: asn-pro-gly. However, the Sma I recognition site has been changed.

Consider another sequence:

```
DNA 5' AAC CCT GGC 3'
```

Again, the amino acid sequence is still the same but the recognition sequence has changed.

DNA Fingerprinting

STUDENT PAGE 3

Activity A: Silent Mutations Questions

1. **Find other changes in the DNA sequence which will still code for the same amino acid sequence, but change the Sma I recognition site.**

2. **Here are two other recognition sequences. Devise your own silent mutations:**

Eco R I (from *E.coli* RY 13) G↓AATTC 3'

Pvu I (from *P. vulgaris*) 5' CGAT↓CG 3'

3. **Explain the relationship between silent mutations and the different sized DNA bands which may be produced from restriction enzyme digests.**

DNA Fingerprinting

STUDENT PAGE 4

Activity B: Pre-lab Exercise for Kit #102

(Consult the "Background Information" sheet from the kit to answer these questions).
Answer on a separate sheet.

1. What is the objective of this lab?

2. List the contents of each tube (A,B,C,D,E,F,G,H).

3. Define: restriction enzyme

4. Recognition site:
   a. Define
   b. Describe each type of recognition site and give an example.
      1. Symmetrical
      2. Cuts are opposite each other
      3. Degenerate

5. Briefly explain the conditions affecting the probability that a given recognition site will occur.

6. Agarose gel electrophoresis
   a. What is it?
   b. Why does DNA migrate toward the positive electrode in an electrophoresis chamber?
   c. On what basis are molecules separated?
   d. Explain briefly:
      - supercoiled DNA
      - nicked DNA
      - catenanes
      - dimer, trimer
   e. Rank the migration of the above forms as they might move in a gel. List from fastest to slowest.

7. Description of the samples you will analyze:
   a. Supercoiled plasmid DNA:
      number of base pairs?
      number of restriction sites for Bgl I ? Eco RI ?
   b. Lambda, a bacteriophage virus (This is a linear DNA.):
      number of base pairs?
      number of recognition sites for EcoRI?
   c. Bovine chromosomal DNA:
      number of base pairs?
      number of recognition sites for Eco RI?

8. Details for procedures (consult "Pre-lab and Special Instructions"):
   a. What is the gel concentration % of agarose?
   b. What is the running time for M20 chamber?
   c. How long to stain with methylene blue?
   d. How long to destain in distilled water?

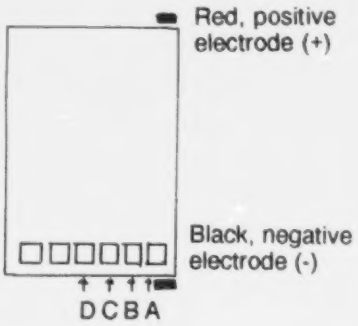
DNA Fingerprinting

STUDENT PAGE 5

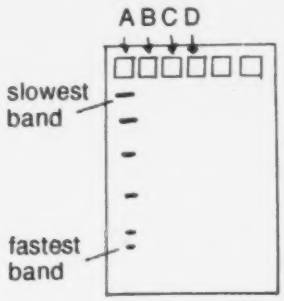
Activity B: Post-lab Analysis for Kit #102

This is your analysis and final write-up sheet.

Load the gel in this positive electrode orientation. (Our apparatus has 10 wells.)



To read the gel, we rotate the stained gel around:



For analysis, observe the original gel. However, consult your copy of the original gel to complete analysis. Then write your analysis in space provided.

Consult Pre-lab Exercise # 2 and #6 d, e. Your interpretation should include reason(s) for the number of bands which appear in the gel and, when appropriate, the relative size of the band as determined by its position on the gel.

Tube A
Contents:
Number of bands: _____
Your interpretation:

Tube B
Contents:
Number of bands: _____
Your interpretation:

DNA Fingerprinting

Activity B: Post-lab Analysis continued

Tube C
Contents:
Number of bands: _____
Your interpretation:

Tube D
Contents:
Number of bands: _____
Your interpretation:

Tube E
Contents:
Number of bands: _____
Your interpretation:

Tube F
Contents:
Number of bands: _____
Your interpretation:

Tube G
Contents:
Number of bands:_____
Your interpretation:

Tube H
Contents:
Number of bands: _____
Your interpretation:

Conclusions

Make a general statement (for example, "I learned that. . ."):

DNA Fingerprinting

STUDENT PAGE 7

Activity C: Background Reading

What Is DNA Fingerprinting?

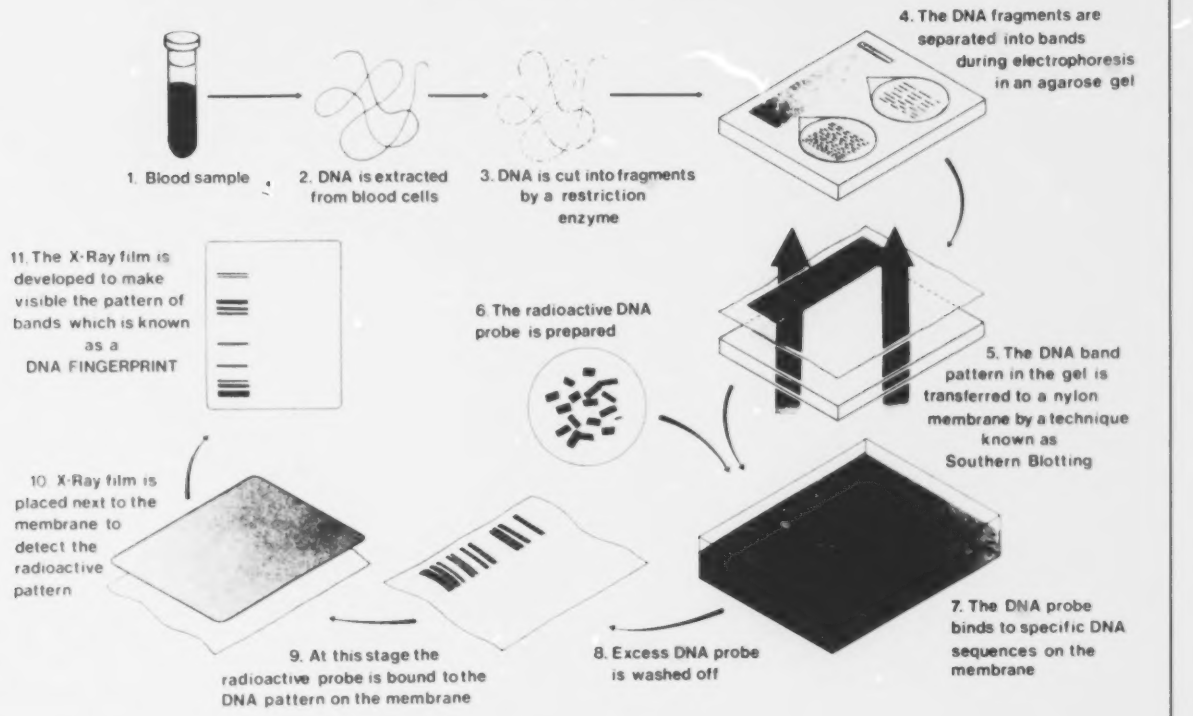
Process

DNA fingerprinting is a technique which is being used to identify the source of human DNA in paternity cases and criminal cases. Sources of DNA samples include not only blood but also skin, semen or hair roots which are used in forensic cases. In criminal cases, analyses would be performed on DNA samples from the crime scene and on suspects. A search for matching band patterns would be made. Positive results can be used as evidence in court if the laboratory has followed a strict chain of custody procedures. In a paternity case a comparison is made between the child's DNA and both the parents. Bands in the child's DNA must be found in either the mother's or father's DNA.

The DNA sample is digested with restriction enzymes and the resulting fragments are separated by agarose gel electrophoresis. If the gel were stained, the fragments would appear as a smear because of the large size of the human chromosome and the many recognition sites of the restriction enzymes.

Consequently after electrophoresis, a procedure known as a Southern Blot is performed. The double stranded DNA fragments are treated with an alkali which separates them into single stranded DNA. The fragments are then transferred to a nitrocellulose sheet (blotting) and incubated with a radioactive (or labeled) DNA probe. The labeled probe is a single stranded DNA fragment complementary to repetitive sequences scattered throughout the genome. These probes will hybridize or bind to their complementary bases, the complementary repetitive sequences. After washing to remove excess probe, the sheet is placed on X-ray film whereupon the hybridized fragments will appear as bands. This process is known as autoradiography. See diagram below (courtesy of CELLMARK Diagnostics).

THE DNA FINGERPRINTING PROCESS


1. Blood sample
2. DNA is extracted from blood cells
3. DNA is cut into fragments by a restriction enzyme
4. The DNA fragments are separated into bands during electrophoresis in an agarose gel
5. The DNA band pattern in the gel is transferred to a nylon membrane by a technique known as Southern Blotting
6. The radioactive DNA probe is prepared
7. The DNA probe binds to specific DNA sequences on the membrane
8. Excess DNA probe is washed off
9. At this stage the radioactive probe is bound to the DNA pattern on the membrane
10. X-Ray film is placed next to the membrane to detect the radioactive pattern
11. The X-Ray film is developed to make visible the pattern of bands which is known as a DNA FINGERPRINT

DNA Fingerprinting

STUDENT PAGE 8

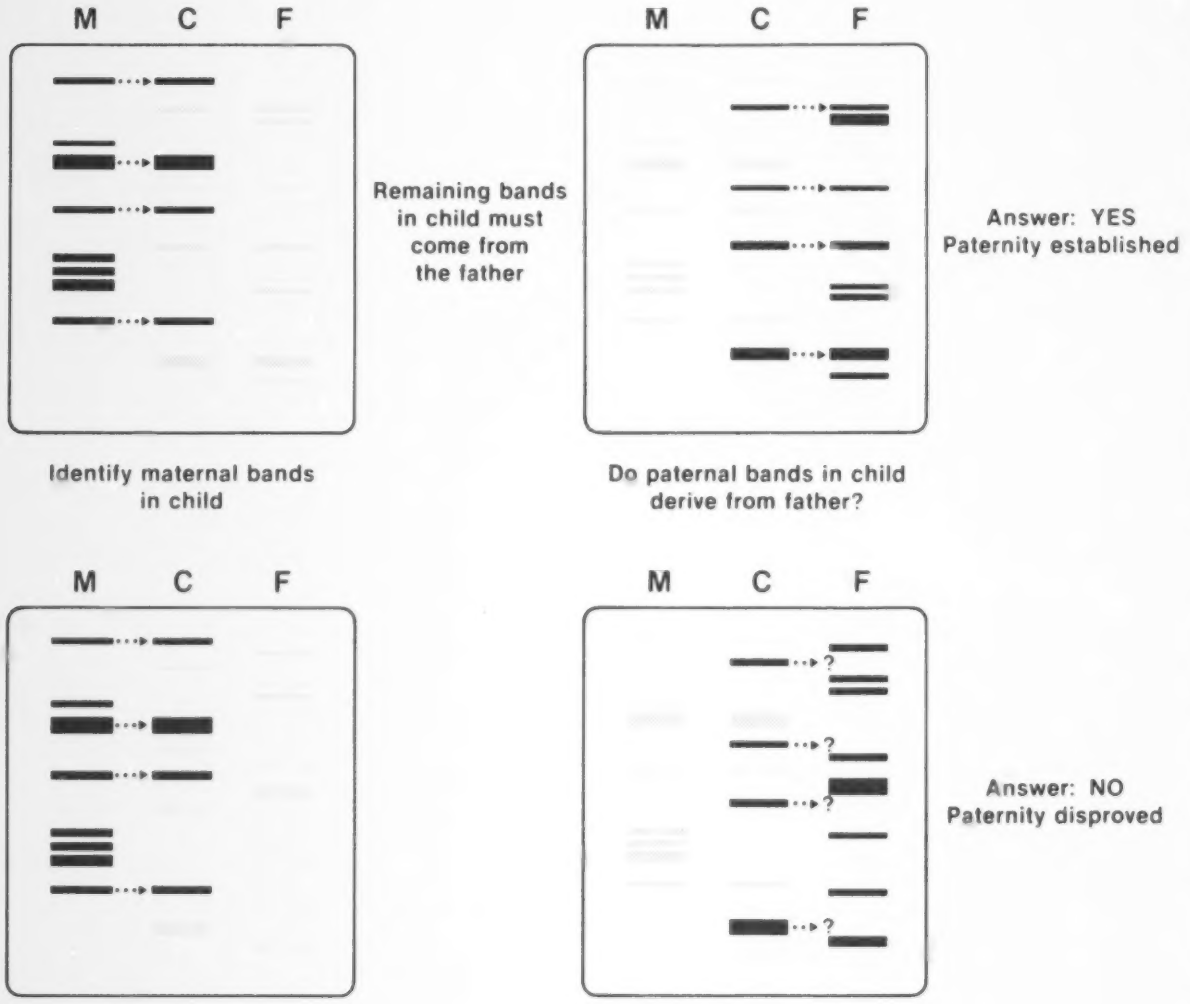
Activity C: Background Reading continued

Analysis

The bands which appear on the developed X-ray film are known as a DNA fingerprint. They resemble the bar code or series of stripes often found on grocery products or boxed merchandise. Each band is the result of the complementary binding of a radioactive probe.

Paternity testing can be determined by first identifying the bands that mother and child have in common. Then the bands remaining in the child's lane must be found in the father's lane. See diagram below (courtesy of CELLMARK Diagnostics).

Analysis of DNA Fingerprints in Paternity Testing


M C F
Identify maternal bands in child
Remaining bands in child must come from the father
M C F
Do paternal bands in child derive from father?
Answer: YES Paternity established
M C F
M C F
Answer: NO Paternity disproved

DNA Fingerprinting

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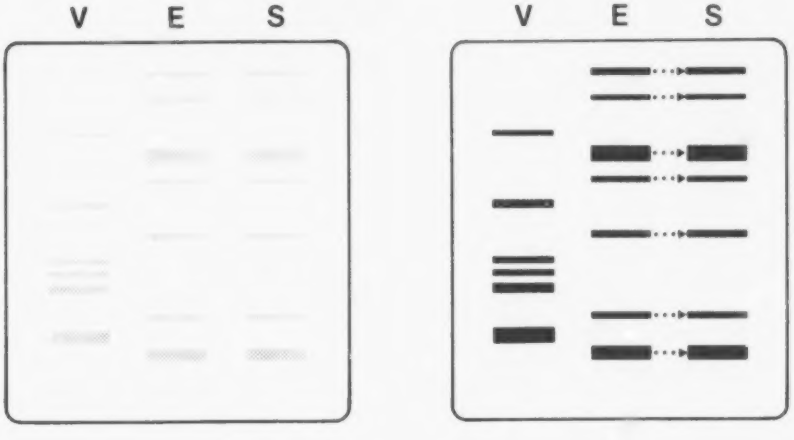
Activity C: Background Reading continued

For forensic testing, the samples to be compared would come from the victim, the crime scene and the suspect(s). If there is a match in band patterns between the suspect and the sample found at the crime scene, this can be used in a court of law. Consult diagram below (courtesy of CELLMARK Diagnostics).

ANALYSIS OF DNA FINGERPRINTS IN FORENSIC TESTING

Answer:

NO MATCH



Answer:

MATCH

LEGEND

V = Victim

E = Evidence

S = Suspect

DNA Fingerprinting

STUDENT PAGE 10

Activity C: Pre-lab Exercise for Kit #109

(Consult "Background Information" included with the kit and the "What Is DNA Fingerprinting?" reading to answer these questions).
Answer on a separate sheet.

1. What is the objective of this lab?

2. Tube identification: List the contents of each tube (A,B,C,D,E,F)

3. What does DNA Fingerprinting involve?

4. **a.** DNA in the average human chromosome contains how many base pairs?
   b. Eco RI might cut human DNA into how many fragments?

5. Explain briefly why no two individuals have exactly the same pattern of restriction enzyme recognition sites.

6. Explain how a "silent mutation" can eliminate a recognition site but leave the protein product unchanged.

7. What effect do intervening repetitive base sequences have on human chromosomes?

8. What are "RFLPs"?

9. List the sources of DNA samples used in forensic cases.

10 **a.** Explain what a probe is in forensic RFLP analysis:

   b. Describe the function of a probe in a Southern Blot.

11. Briefly describe the scenario you are about to analyze with the help of DNA fingerprinting logic.

12. Details of the procedure:
    What is the agarose gel concentration?
    What is the running time for an M 20 apparatus?

DNA Fingerprinting

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Activity C: Post-lab Analysis for Kit #109

Observe the original gel.
Consult your copy of the original gel.

1. List contents of each lane.

   Lane 1:

   Lane 2:

   Lane 3:

   Lane 4:

   Lane 5:

   Lane 6:

2. Which individual, suspect #1 or suspect #2, was at the crime scene? Explain your answer.

3. Could the DNA samples have been distinguished from one another if only EcoRI had been used? Explain your answer.

4. Conclusions ("I learned that. . ."):

Selecting Soil Organisms

Ability level .....Grades 7 – 12
Class periods required 2

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Introduction

The purpose of this lab is to show that many of the enzyme systems needed for the clean-up of society's wastes already exist in nature among the decomposers. Students learn how to extract and grow bacteria from the soil and test it for the ability to digest starch.

This activity could fit into a unit on ecology or microbiology as well as biotechnology.

Objectives

At the end of this activity students should:

1. Be aware of the existence of dormant microbes in soil and water.
2. Know that specific decomposers can be isolated by using selective media.
3. Realize that scientists are searching for natural enzyme systems capable of biodegrading society's wastes.
4. Be able to define "bioremediation."

Materials

For entire class:

- Bunsen burners
- balances
- autoclave or pressure cooker

For each team

- nutrient agar containing 0.5% soluble starch
- Gram's iodine
- bacterial spreader or loop
- 2 Petri dishes
- 2 sterile 1 ml pipets
- 2 sterile capped bottles or foil wrapped test tubes
- sterile water
- 95% ethanol

Teacher Preparation

1. Make starch agar:
   a. Add 2.0 g of soluble starch to 400 ml of distilled water.
   b. Add 9.2 g of nutrient agar and autoclave 15 minutes at 121°C (15 psi of steam pressure). This will make 20 plates.
   c. Sterilize water and glassware using same procedure. (Foil wrapped glassware can also be sterilized in the oven at 400 F for 2 hours).
2. Make Gram's iodine:
   Add 1.0 g of iodine and 2.0 g of KI to 100 ml of distilled water. (Lugol's iodine can also be used.)

Note: *The procedures above could be done without using sterile water and dilution bottles. However, in order to show that the organisms are coming from the sample and not contamination, sterile technique is desirable for the remainder of the procedure.*

3. Have each team bring in a soil sample:
   Soil samples should come from varying environments such as an open field, forest floor or near garbage cans. A water sample from a pond or ditch can be substituted for a soil sample. The soil or water sample will be diluted from 1:10,000 so the effect of individual colonies can be seen on the plate.
4. Put class data chart on the board or overhead (see example below).

Group #	Source of Soil or Water Sample	# of Colonies

Teaching Tips

1. Methods for sterile technique, serial dilutions and pipetting may need to be reviewed.
2. If students have difficulty with the measurement of 0.1 ml you may want to premeasure the inoculum for them.
3. Organisms can be disposed of by soaking the plates in a 20% chlorine bleach solution overnight.
4. Ideas for extending this activity:
   a. Colonies can be isolated from the plates (has to be done immediately after the iodine is poured to prevent killing the colonies), replicated and grown on differential media for identification.
   b. Letting the plates grow for longer than 48 hours until all the starch is digested will illustrate the concept of carrying capacity.

Procedure

(See student pages for detailed protocol.)

Students do the following:

1. Weigh out soil and dilute with sterile water.
2. Inoculate a petri dish with 0.1ml of the soil dilution. (Using 0.1ml

Selecting Soil Organisms

instead of the standard 1 ml dilutes the sample an additional tenfold.)

3. Invert the plates and wait overnight
4. Examine the plates for clear areas. (Should be done the next day so that the enzyme does not become diffused throughout the plate.)
5. Test the plates for starch with iodine.
6. Draw the location of starch-digesting bacterial colonies.
7. Summarize class results (see figure 2 for chart).
8. Answer questions.

Answers to Student Questions

1. *When the bacteria digests the starch, the agar appears clear rather than blue when stained by iodine.*
2. *amylase*
3. *By forming resistant endospores*
4. *If your purpose is to select for oil-digesting bacteria, a lightweight oil such as sewing machine oil could be used in place of starch agar.*
5. *Because oil is a by product of decomposition by microorganisms*
6. *To eliminate nutrients as a limiting factor in the growth of the bacteria*
7. *Many of the organic products synthesized by man are unique and the metabolic pathways necessary for their decompostion have yet to evolve.*
8. *Waste products often require several metabolic pathways to decompose this mixture of chemicals. A community of microorganisms would provide the required multiple enzymes system.*
9. *If several metabolic pathways could be engineered into a single organism only one organism would be required to decompose this waste product.*

Resources

Aaronson, S. (1970). *Experimental microbial ecology*. New York: Academic Press.

Begley, S. & Waldrop, T. (1989, June 19). Microbes to the rescue. *Newsweek*, pp. 56-57.

Selecting Soil Organisms

STUDENT PAGE 1

Background Reading

Introduction

The purpose of this lab is to show that many of the enzyme systems needed for the clean-up of society's wastes already exist in nature among the decomposers. Using bacteria (and fungi) to biodegrade waste is called *bioremediation*. Humans have used bacteria in sewage treatment plants and in composting for many years. Environmentalists would like to see microbes used to decompose unwanted oil resulting from oil spills and contaminants in ground water. There is no need for man to insert a gene into a bacterium to clean up waste material if there is already a bacterium in nature that can do the job. Additionally, state and federal regulations presently restrict the release of genetically engineered "bugs" for waste management. In this lab you will simulate the process of selecting a bacterium from nature that can degrade a particular substance. We will use starch instead of a toxic subtance such as oil. You will look for bacteria that have the enzyme *amylase*, which makes them capable of digesting starch.

Selecting Soil Organisms

STUDENT PAGE 2

Activity Sheet

Materials

For each team

- nutrient agar containing 0.5% soluble starch
- Gram's iodine
- bacterial spreader or loop
- 2 Petri dishes
- 2 sterile 1 ml pipets
- 2 sterile capped bottles or foil-wrapped test tubes
- sterile water
- 95% ethanol

Procedure

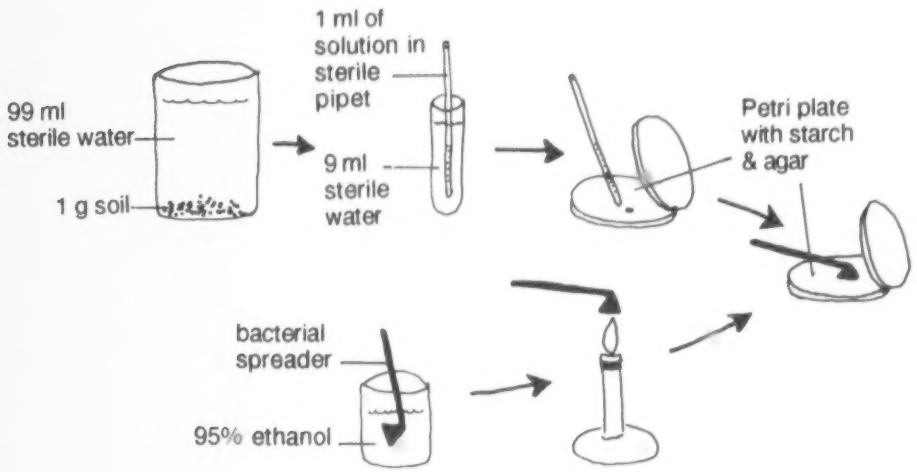
(See diagram.)

1. Weigh out 1 gram of soil and add this to 99 ml of sterile distilled water. **Dilute** this further by removing 1 ml of this mixture and adding it to another bottle which contains 9 ml of sterile distilled water. Mix by swirling. *(Make sure containers are sterile and capped so that the soil can be mixed thoroughly.)*

2. Remove 0.1 ml of this mixture and put on a petri plate containing starch and agar. Dip a bacterial spreader or inoculating loop in alcohol, hold it in a flame briefly and cool it on the edge of the agar plate. Use this to spread the 0.1 sample evenly around the plate. Repeat this with a second plate.

3. Turn the plates over and leave overnight at room temperature.

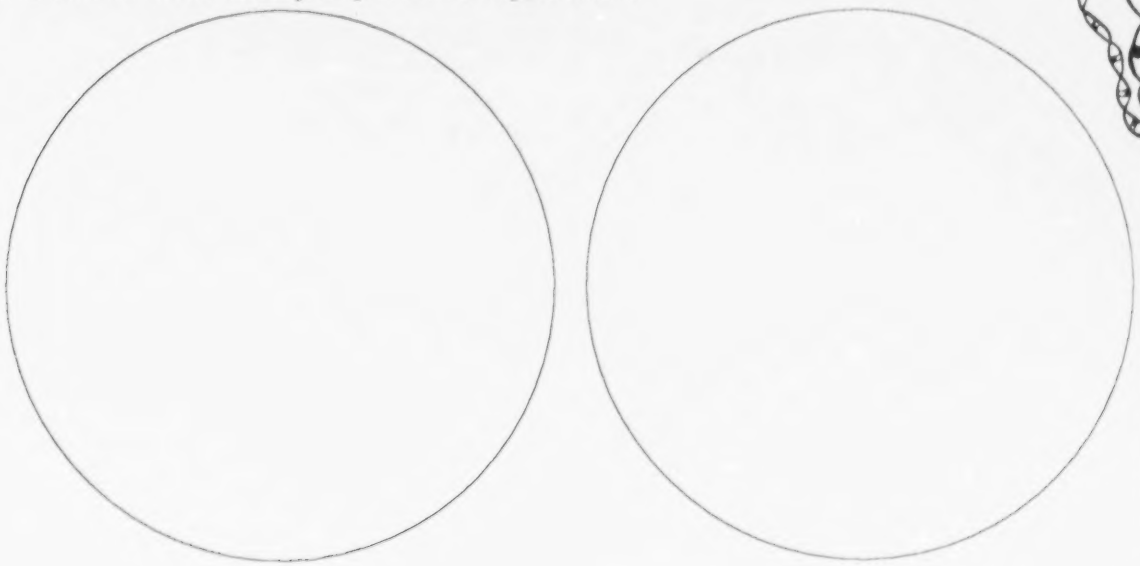
4. The next day, examine the plates to see if clear areas are developing in the starch agar. If clear areas are seen, flood the plate with the iodine mixture. Pour off the excess iodine after 1 minute. Bacterial colonies capable of digesting starch will be surrounded by a brown "halo."


1 ml of solution in sterile pipet
99 ml sterile water
1 g soil
9 ml sterile water
Petri plate with starch & agar
bacterial spreader
95% ethanol

Selecting Soil Organisms

STUDENT PAGE 3

5. Draw in the colonies on your plates in the space below:



6. Enter your results on the Class data chart.

7. Follow your teacher's directions for plate disposal.

Class Discussion

Determine which types of samples produced starch-digesting bacteria.

Were there problems which prevented some groups from isolating any bacteria?

What other enzymes could be selected for in a similar manner (lipase, cellulase, etc.)?

Selecting Soil Organisms

STUDENT PAGE 4

Questions

1. What were the clear areas on the plates?

2. What enzyme do starch digesting bacteria contain?

3. How could bacteria survive in soil or water which did not contain starch?

4. How could this method be used to isolate oil degrading bacteria?

5. Why do oil digesting decomposers exist in nature?

6. When Exxon tried to clean up the oil spill in Prince William Sound, why did they add fertilizer to the oil?

7. Why have we been unable to find bacteria or fungi which are capable of digesting many of our waste products?

8. Companies that specialize in cleaning up toxic waste spills use a mixture of bacteria and fungi rather than a pure culture. Why is this micro-ecosytem approach more effective?

9. Biotechnologists are in the process of engineering "super bugs" to clean up toxic wastes. Why have they been unable to release these organisms into the environment?

10. Describe a situation in which a genetically engineered decomposer might have an advantage over a natural mixed culture.

Molecular Basis of Cancer

Ability level ..... Grades 7-12 general biology
Class periods required 1

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Introduction

In this lesson students build cell models out of plastic foam, pins and beads in order to understand the relationship between changes in gene composition and metabolic activity in a cell. If the genetic change and the resulting change in the metabolic activity interrupt the regulation of cell division, cancer may result. Experimental cancer treatment includes the precise delivery of immunotoxins to cancerous cells. Immunotoxin (monoclonal antibody plus cytotoxin) is a recently developed biotechnology procedure.

Objectives

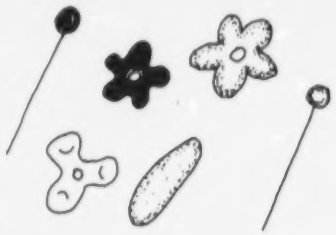
At the end of this activity, students should:

1. Understand how the response of cells to the environment depends on receptors and antigens on the cell surface.
2. Understand that genes are responsible for coding for receptors and antigens.
3. Understand that a change in the DNA affects a change in the gene, the receptor, and therefore a change in cell behavior.
4. Know that when a change occurrs in the receptors that regulate cell division, cancer may result.
5. Know that a single mutation does not cause cancer.
6. Know that one experimental cancer treatment involves production of immunotoxins which bind to the unique antigens produced by the cancer cells.
7. Recognize and understand these words: **antigen/antibody, oncogene, receptor, immuno-toxin, monoclonal antibody, mutation, protein synthesis.**

Materials

Per student

- 2 plastic foam balls
- 20 (approx.) plastic beads (see diagram)
- 10 (approx.) long straight pins (longer than the longest bead)



Teacher Preparation

Obtain materials (craft and hobby shops will have them).

Teaching Tips

After students complete the activities you may wish to use the following as discussion topics:

1. Research has shown that immunotoxin is more successful in liquid tissue cancer than solid tissue cancer. Discuss plausible reasons for this event.
2. Some mutated receptors do not lead to cancer. Discuss other cell fates from mutated receptors.

Procedure

Activity I

Students do reading and answer questions on worksheet.

Activity II

Students build a normal cell model and answer questions on worksheet

Activity III

Students build another cell model showing changes in the receptors and antigens.

Activity IV

Class discussion of questions from the worksheet.

Answers

Activity I

1. a. *UUG AUC CCU GGU*
   b. *leucine-isoleucine-proline-cysteine*
   c. *The second amino acid would be different, which changes the nature of the protein product.*

Activity II

1. *It shows volume, surface has different protein molecules embedded in it, each cell has its unique antigens.*
2. *Genes*
3. *They receive a hormone which causes a chemical reaction to occur inside the cell that is associated with regulation of cell metabolism. This, in turn, affects the activity of regulatory proteins for growth. If the receptor receives the right hormone signal, then regulation is working.*
4. *The receptors that regulate cell division*
5. *The antibodies produced by the immune system will attack the cell.*

Activity III

1. *Nucleus*
2. *Mutation*
3. *It shows one mutation on one allele or co-expression of the normal and mutated allele.*

Molecular Basis of Cancer

4. *A gene of the pair is mutated at a different place or type.*
5. *The gene is gone (broken chromosome) or the gene has been altered so that the receptor produced will not fit in the membrane.*
6. *Another mutation event may back mutate or revert to normal.*
7. *Normal genes gone wrong*
8. *Single base substitution to more drastic changes in DNA*
9. *Radiation, environmental chemicals, smoking (discuss how these agents react chemically with DNA)*
10. *Several different genes must mutate to bring about malignancy.*
11. *Constant exposure to mutagenic agents*

Activity IV

Class discussion questions

1. *Any molecule that induces an immune response or antibody production*
2. *Produced from mutated genes*
3. *The immune system produces antibodies and destroys the unlike cells.*
4. *No, usually cancer cells are destroyed by the body's immune system .*
5. *Monoclonal antibodies are produced that will seek out and attach to the antigens on cancer cells but not the healthy neighboring cells.*

Additional Activity

Have students research the production of monoclonal antibodies.

Resources

Campbell, N.A. (1987). *Biology* Menlo Park, CA: Benjamin/Cummings Publishing Company.

Curtis, H. (1989). *Biology*. New York: Worth Publishers.

Molecular Basis of Cancer

STUDENT PAGE 1

Activity I. Reading

What Is Cancer?

Cancer cells have lost control of their own reproduction and do not respond to normal controls on growth. Normally, hormones from different places in the body interact with receptors on the cell surface and stimulate cell division. The genes inside cells contain instructions for making these hormone receptors. Sometimes the substitution of a single base in a gene is enough to alter the receptors and prevent the cell from responding to hormone signals.

Genes that result in cancer are called ***oncogenes***. Scientists believe that cancer is a result of cumulative changes in DNA: It takes several oncogenes working together to bring about a malignancy. The first oncogene frees the cell from normal limits on reproduction. The second oncogene brings about rapid cell division and other physical changes characteristic of cancer. Oncogenes also code for production of abnormal ***antigens*** which are incorporated in the cell membrane. Normal antigens identify cells as belonging to the body. Strange antigens signal the body's immune sytem that a foreign invader is present. Scientists don't understand why the immune system doesn't always respond to the abnormal antigens on cancer cells. When cancer cells are allowed to grow the result is a malignancy.

Biotechnology researchers are using this knowledge in searching for a "magic bullet" to selectively destroy cancerous tissue. The abnormal cancer antigens can be used to make ***monoclonal antibodies*** that can be produced in large quantities in a laboratory, then injected into the patient. These antibodies are mixed with a cell poison to make an ***immunotoxin***. When given to a patient, the immunotoxin finds and selects cells with the cancer altered antigen and subsequently binds to it. The target cells then die as a result of the poison which the antibody has delivered to the cell. Patients with cancers of the blood (leukemia) or lymph nodes or system (lymphoma) have experienced some success with this experimental treatment. The use of immunotoxins is preferable to traditional chemotherapy because doctors can use a much lower dosage, and there are fewer side effects.

Molecular Basis of Cancer
STUDENT PAGE 2

Activity I. Questions

1. In a hypothetical segment of one strand of a DNA molecule, the sequence of bases is:

(3') A A C T A G G G A C C A (5')

a. What would be the sequence of bases in an mRNA strand transcribed from this DNA segment?

b. What would be the sequence of amino acids coded by the mRNA?

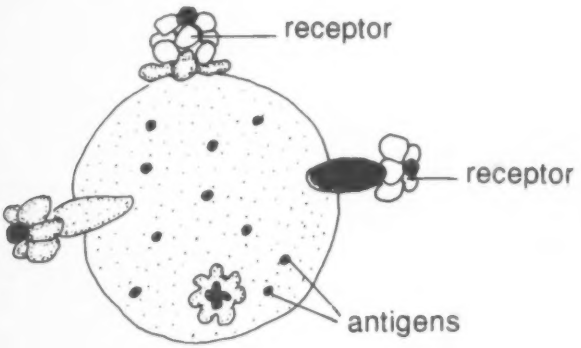
c. If cytosine is substituted for thymine in the DNA, what change would occur in the amino acid sequence?

m-RNA	amino acid
AAC	tyrosine
UUG	leucine
UAG	stop
AUC	phenylalanine
GGA	stop
CCU	serine
CCA	serine
GGU	cysteine

Molecular Basis of Cancer
STUDENT PAGE 3

Activity II. 1st Model

Build a model of a normal cell using a plastic foam ball and plastic decorations. The decorations represent normal receptors found on the cell surface. Straight pins (with no decorations) represent other antigens. Make as many different receptors as possible. See diagram below for an example of a normal cell:



Questions

1. In what ways does this model resemble a real cell?

2. What makes the different receptors?

3. What is the job of the receptors?

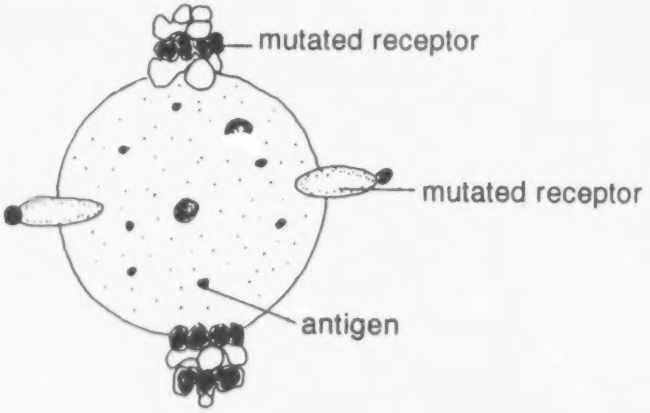
4. Which receptors are involved with cancer?

5. The body's immune system does not react to antigens of normal cells. What happens when the antigens are changed?

Molecular Basis of Cancer
STUDENT PAGE 4

Activity III. 2nd Model

Build another model showing changes in the receptors and antigens. Use the same number of receptors as in the normal model. An example of this cell might be:



Observe the changes in the models

Questions

1. The changes appear to have occurred on the cell surface only. Where did the change actually occur?
2. What caused the receptors to change?
3. If the two receptors were alike in the first model and the second model shows only one change, how can you account for the change?
4. If two receptors were alike in the first model, and both show a different change in the second model, how can you account for the change?
5. If the two receptors are completely missing, how can you account for the change?
6. Can a mutated gene be changed to normal?
7. What is an oncogene?
8. What can change normal genes to an oncogene form?
9. What are some mutagenic agents?
10. Why does the incidence of cancer increase with age?
11. What can an individual do to increase the odds of getting cancer?

Molecular Basis of Cancer
STUDENT PAGE 5

Activity IV. Discussion Questions

1. What are antigens?

2. Why are cancer cell antigens different from normal cells?

3. How does the body combat cells with foreign antigens?

4. Does every cancer cell develop into a malignancy?

5. How is biotechnology used in cancer treatment?

Applying Principles of Ethics to Bioethical Decision Making

The lessons in Unit V may require some discussion of ethics as it relates to the implementation of biotechnology. For many teachers this may be a previously untrodden landscape. The following is a brief introduction to the complex field of ethics.

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Ethics and Science share a commitment to rational thinking. The scientific method is a form of rational inquiry into **facts**. We ask: What are the facts and can we explain them using a rigorous application of the rules of the scientific method? Ethics is a form of rational inquiry into **right and wrong actions.** We ask: What is the right thing to do and can we justify our decision using the application of ethical principles?

Those who study ethics have identified three guiding principles as the foundation for ethical decision making:

1. Beneficence

The principle of beneficence states that we should promote good for others and avoid harm to them. This principle can be applied to whole groups of people as well as individuals. The application of this principle in bioethical decision making requires us to ask: will our decision result in a greater balance of good over harm? For example, critics of the Human Genome Sequencing Project have raised several questions requiring the application of the beneficence principle. These questions include: Whose genes will be sequenced? Will this project result in any human suffering? Will there be a greater good for all as a result of this project? Can the information resulting from the genome project be used against people?

2. Respect for Autonomy

The core of this principle is the intrinsic value of the individual. Each person has fundamental self worth and dignity which should be respected by others, even if they hold different values. In addition, individuals should have freedom to control their lives. Using the Human Genome Project as an example, questions that require consideration of this principle are: Will the information gained from this project have an impact on the freedom of individuals to determine career choices? How will the information be used by health care professionals in determining treatment? Will there be any limits on personal decision making?

3. Justice

There are three ways to approach the principle of justice. The first is that everyone has an equal right to basic needs - food, shelter, clothing, health care, education, employment. The second is that some individuals have earned the right to special treatment based on their merit. A third approach is to treat everyone equally regardless of perceived merit. When applying the principle of justice one can either look at the outcomes of a decision or consider the process leading to the outcome while remaining neutral as regards the outcome itself. Questions about the Human Genome Project which can be considered using this principle are: What is the most equitable way to distribute the funds earmarked for scientific research? On the basis of contribution to society? Merit? Best use of resources?

If values are going to be included and discussed in the classroom, teachers and students should have a basic understanding of the principles of ethics. Otherwise debates and discussions become "free-for-alls" leaving students with the conclusion that the issues are irresolvable and that only experts can make decisions.

The following sources were helpful in the development of this introduction:

Candace C. Gauthier, University of North Carolina, Wilmington.

George Kieffer, University of Illinois at Urbana-Champaign.

Advances in Genetic Technology - teacher's edition. (1989). BSCS, D.C. Heath and Company.

Principles of Biomedical Ethic., (1989). Beauchamp and Childress, Oxford Press.

Debating Bioethics

Ability level ..... Grades 7-10
Class periods required ..... At least 1 for 1 debate; at least 2 homework assignments for student prep

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Introduction

Bioethical decisions are difficult because they often require specialized knowledge about a new procedure or technology. Examples of this include recent questions about the intentional release of bioengineered organisms, human gene therapy, use of bovine growth hormone, transgenic animals, patenting genetically engineered plants and animals, screening for genetic diseases and sequencing the human genome. This activity uses debates to teach some specialized knowledge about biotechnology as well as introduce students to the ethical questions that new technologies can raise.

Objectives

At the end of this lesson, students should be able to:

1. Understand some of the ethical questions related to biotechnology.
2. Use critical thinking skills to analyze and answer complex ethical questions.
3. Be able to use and understand the following key words: **bioethics, claims, values, policy, debate**.

Materials

Literature concerning bioethical issues (books, articles, pamphlets).

Teacher Preparation

Assemble reference materials. Newspapers and magazines such as *Discover* and *Scientific American* are good sources for articles.

Teaching Tips

1. You may want to review the principles of ethics (described at the beginning of Unit V) and methods of debating with your students.
2. Invite students from other classes in for the debate. Devise a pre and post debate opinion sheet for the visitors to record their opinion about the debate topic. Use these sheets to determine the effectiveness of the debate team's persuasion. This technique could also be used with the class as a way to keep everyone involved during the debate.

Procedure

1. Discuss possible debate topics with your class.
2. Divide the class into groups of four and have them choose a topic of interest to them. Allow the group members to decide who will be pro and con.
3. Give the students some time to research their topic and prepare for the debate. (This will require homework assignments.)
4. Students should use the information on the student pages (including the Bibliography) to help them prepare for the debate.
5. Follow the directions on the student pages for the debate format.

Resources

See bibliography provided as student pages for printed materials.

Computer Software:

Can You Own Life? Patenting Genetically Engineered Animals. (1988). Learning Designs, Inc, Social Science Education Consortium, Boulder CO. Requires a Macintosh Plus, SE or II computer with HyperCard. This program uses HyperCard stacks and an excellent exercise in bioethical decision making from a social studies perspective. It is extensive and would require a sizable block of time to implement in the classroom but might be used in a co-teaching relationship with a science and social studies teacher. It is appropriate for middle and high school students.

Debating Bioethics

STUDENT PAGE 1

Preparing for the Debate

Your group should pick a topic and consider some of the ethical issues within that subject. Then use the information in "Making a Claim" to help you formulate a claim that you can debate.

Possible Topics

- Intentional release of engineered organisms
- Human gene therapy
- Use of bovine growth hormone
- Transgenic animals
- Patenting genetically engineered plants and animals
- Screening for genetic diseases
- Sequencing the human genome

Example:

Topic: *Genetic Screening*

Ethical issues to consider

1. An expectant mother and father are told that their future child carries a gene for a genetic disorder. What options are or should be available to them?
2. Will future laws give employers the power to genetically screen each new employee for a genetic disorder? If the test proves to be positive, will the employer have the right not to hire the individual because of some future health benefit costs that may eventually be paid out to the employee?
3. Should genetic screening be used to refuse entry of an individual into the United States?
4. Should genetic screening be required for obtaining life insurance? If an individual tests positive for a genetic disorder, will he/she be required to pay a higher premium? Will the insurance company have the right to refuse or cancel his/her application for life insurance?

Possible claims to debate

claim of fact ..... Genetic screening is not a reliable predictor of disease.

claim of value ..... Results of genetic screening should be private.

claim of policy ..... Insurance companies should be permitted to require genetic screening.

ME.90

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Debating Bioethics

STUDENT PAGE 2

Making a Claim

The Claim

All argument or debate is developed from a claim. The claim answers the question "What are you trying to prove?" There are three main kinds of claims.

Claim of Fact

This is supported by facts or data such as statistics, graphs and research findings from reliable and reputable sources which the audience can accept. An example of this type of claim is "Capital punishment is not a deterrent to crime."

To defend a factual claim you should:

- Be sure that what you are trying to prove is clearly stated from the beginning.
- All terms that might be controversial or ambiguous (i.e. "surrogate mother") should be defined at the start.
- Present data that is sufficient, accurate, recent and from reliable sources. An authority with credentials from Harvard University who published an article in *Scientific American* will have higher credibility with an audience (and your teacher) than a citation from an article in the *National Enquirer*.

Claim of Value

This attempts to prove that some things are more or less desirable than others based on standards of taste and morality. Claims of value express approval or disapproval, and require the audience to make a judgment. Advertisements are common sources of value claims, but value claims emerge whenever people argue about what is good or bad, beautiful or ugly. An example of this type of claim is,"Using animals for research is wrong."

To defend a claim of value you should:

- Prove the values or principles to be more important than the end result.
- Use examples and illustrations to clarify meanings and make distinctions.
- Use the testimony of experts to prove that knowledgeable or highly regarded people share these values.

Claim of Policy

This asserts that specific policies should be instituted as solutions to problems. The expressions "should, must or ought to" are usually used. An example of this type of claim might be, "Using microorganisms for biological warfare must be stopped."

To defend a claim of policy you should:

- Make your proposal for change clear, establishing the distinct benefits gained by adopting your proposal.
- Support your proposal with solid data, moral considerations and common-sense reasons.

Debating Bioethics

STUDENT PAGE 3

The Debate

Format

- 5 minutes pro
- 5 minutes con
- 2 minutes for consultation
- 2 minutes rebuttal con
- 2 minutes rebuttal pro

Grading

Each person will be given a grade for the in-class presentation.

Criteria include:

1. Clear, concise presentation of position (15 points).
2. Substantive foundation from the literature for argument (30 points).
3. No overlap between presenters on same side of issue (10 points).
4. Rebuttal addressing issues raised by opponents–not rehash (20 points).
5. Clear presentation of scientific issues (10 points).
6. Persuasiveness (15 points).

Debating Bioethics

STUDENT PAGE 4

Bibliography

Advances in genetic technology. (1989). Lexington, MA: D.C. Heath – Biological Sciences Curriculum Study.

Armstrong, S. (1986, October). The business: Genetic engineering. *Christian Science Monitor*, pp. 20-21.

Basic genetics: A human approach. (1983). Colorado Springs, CO: Kendall/Hunt – Biological Sciences Curriculum Study.

Benbrook, C.M. & Moses, P.B. (1986, November/December). Engineering crops to resist herbicides. *Technology Review*, pp. 55+.

Biomedical technology. (1984). Colorado Springs, CO: Kendall/Hunt – Biological Sciences Curriculum Study.

Brill, W. J. (1986, October). Biotechnology: Its potential. *EPA Journal*, p. 6.

Brozan, N. (1988, January 24). Egg donations: Miraculous or immoral? *Providence Sunday Journal*, p. B1.

Carey, J. (1986, November/December). Brave new world of super-plants. *International Wildlife*, pp. 16-18.

Cowen, R.C. (1986, September 26). The science: Genetic engineering. *Christian Science Monitor*, pp. 18-19.

Edwards, D.D. (1986, January 25). A common medical denominator. *Science News*, pp. 60-62.

Fielding, B. (1985, August). Biotechnology: The promise and the peril. *The Environmental Forum*, pp. 13-20.

Foerstner, A. (1985, October 13). Boy or girl?: Science helps parent pick infant's sex. *Chicago Tribune*, Section 3, pp. 17+.

The genome initiative. (1987, August 31). *Newsweek*.

Gene and surroundings. (1983). Colorado Springs, CO: Kendall/Hunt – Biological Sciences Curriculum Study.

Grady, D. (1987, June). The ticking of a time bomb in the genes. *Discover*, pp. 26.

Hotz, R.L. (1987, May 10). Secrets of life, part 1: Awesome questions for mankind created for test-tube advances. *Palm Beach Post*, p. 1A+.

Hotz, R. L. (1987, May 11). Secrets of life, part 2: Couple fearful of diseased baby, hunting genetically clean egg. *Palm Beach Post*, pp. 2A+.

Hotz, R. L. (1987, May 12). Secrets of life, part 3: Genetic testing could give employers inside view of workers. *Palm Beach Post*, pp. 8A.

Immunology and human health. (1986). Colorado Springs, CO: Biological Sciences Curriculum Study.

Joyce, C. (1987, March 5). The race to map the human genome. *New Scientist*, pp. 35.

Kolata, G. (1985, December 6). Down syndrome – Alzheimer's linked. *Science*, pp. 1152-1153.

Lander, E.S. (1987, March 25). The new human genetics: Mapping inherited diseases. *Princeton Alumni Weekly*, pp. 10-16.

Lawren, B. (1985, November). 1990's designer beasts. *Omni*, pp. 57+.

Lehman, B.A. (1985, November 27). Boy or girl?: Doctor doesn't leave it to fate. *Miami Herald*, p. 2E. Reprinted copy of Boston Globe.

Lewis, R. (1987, June). Harvesting the cell. *High Technology Business*, pp. 30-33.

Lewis, R. (1987, January). Your next child will be a: Boy? Girl? *Health*, pp. 58+.

Lyon, J. & Gorner, P. (1986, March 2-9). Altered fates: Gene therapy Altars future. *Chicago Tribune*, Tempo, pp. 1+.

Miller, N. (1986, November 23). Amniocentesis: Pre-natal test can be blessing or torment. *Boca Raton News*, pp. 1E+.

Newman, A. (1988, April). The Legacy on chromosome 4. *Johns Hopkins Magazine*, pp. 30-39.

Pimental, D. (1987, January). Down on the farm: Genetic engineering meets ecology. *Technology Review*, pp. 24+.

Predicting diseases. (1987, May 25). *U.S. News and World Report*, pp. 64-70.

Rolbein, S. (1987, September). Braving a new world. *Boston Magazine*, pp. 88+.

Rottenberg, A.R. (1985). *Elements of argument*. St. Martin's Press.

Schmeck, H.M. (1985, November 10). The promises of gene therapy. *New Times Magazine*, pp. 114+.

Scientists set to unravel 80,000 element sequence of human genetic code. (1986, September 7). *Baltimore Sun*, p. 11A.

Schwartz, R. (1985, September). Designer genes at the dinner table. *FDA Consumer*, pp. 31-33.

Some possible uses of biotechnology. (1986, October). *EPA Journal*, p. 13.

Sun, M. (1987, April 3). Biotechnology developments. *Editorial Research Reports*, pp. 154-162.

Thompson, L. (1987, March 16). The race is on to map the gene. *The Washington Post, National Weekly Edition*, pp. 11-12.

Thompson, R. (1986, January 10). Genetic breakthrough. *Editorial Research Reports*, pp. 3-20.

Vine, G. (1987, November 19). Test tube embryos. *New Scientist*, pp. 1-4.

Wolinsky, H. (1988, April). Fetal farms?: Transplants from the unborn. *American Health*, pp. 47.+

Risk/Benefit Analysis

Ability level ..... Junior high, Grade 10 regular biology, AP biology
Class periods required ..... Will vary. At least 1 + a homework assignment

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Introduction

In this activity students look at specific scenarios of people in situations that require a bioethical decision. Using risk/benefit analysis as a critical thinking tool, students try to come to a decision as a group and as individuals. Risk/benefitting is only one means of addressing moral issues. One would hope that ethical decisions are based on more than R/B analysis. However, this method facilitates initial considerations of ethical/moral problems. It involves brainstorming, literature search, small and large group discussion, recognition and weighing facts and opinions, and decision-making skills related to current bioethical issues.

Objectives

At the end of this lesson, students should be able to:

1. Use critical thinking skills in making decisions about environmental, social and moral issues.
2. Conduct library research on a certain topic.
3. Recognize and understand these words: **amniocentesis, cystic fibrosis, Huntington's disease, Duchenne muscular dystrophy, dioxin, chorionic villi biopsy**

Materials

- Copies of student pages (scenarios and risk/benefit analysis form) for each student

Teacher Preparation

1. Read through the scenarios.
2. Photocopy the student pages.

Teaching Tips

A. The scenarios may be assigned to specific groups of students as homework. Students can do library research on the assigned topic.

B. The teacher should serve as a facilitator. He/she should try not to influence student decisions but place questions and information appropriately and insure on-task procedure in a timely fashion.

C. Use a variety of scenarios to address ethical issues in medicine, agriculture, environment, etc.

Procedure

1. A scenario involving a bioethical issue is presented to the class.
2. Students are given the opportunity to share any information or ideas about the issue with the group (i.e. brainstorm).
3. Students use the Risk/Benefit Analysis Sheet to describe the issue and record information upon which to base their decisions.
4. The class discusses the difference between a fact and an opinion.
5. The teacher may choose to share information about the issue and/or assign a search of available literature to gather information.
6. The class shares the information gathered about the topic. Students should list and differentiate facts and opinions as they record information.
7. The class breaks into small, workable groups; assign tasks as follows:
   a. Each group should list the risks or benefits on the issue. OR,
   b. Alternate groups should describe risks or benefits only.
   c. Each group should select a reporter.
8. Students gather together and share the group's risk benefit listings. Address questions as necessary. Each group should present its conclusions that result from weighing the risks and benefits.
9. When discussion is complete, individual students make an ethical decision regarding the scenario and write a supporting statement.

Resource Materials

Dushkin Publishing Group. *Taking sides: Clashing views on controversial bioethical issues*. Guilford, CT. Handled by Grolier Publishing Co.

Norman Fost (consulting ed.). Opposing viewpoints sources science and technology. Greenhaven Press, Inc., 577 Shoreview Park Rd., St. Paul, MN 55126.

Benjamin, M., et al. (1984). Short children, anxious parents: Is growth hormone the answer? *Hastings Center Report, 14*(2), 13.

Brill, W.J. (1985). Safety concerns and genetic engineering in agriculture. *Science, 227*(4685), 381.

Office of Technology Assessment. (1982). Genetic technology: A new frontier. Boulder, CO: Westview Press.

Tangley, L. (1983). Engineered organisms in the environment? Not yet. *Bioscience, 33*(11), 638.

National Science Board. (1984). *Statement on research related to biotechnology*. Washington, DC: National Science Foundation.

President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. (1982). *Splicing life*. Washington, DC: U.S. Government Printing Office.

Office of Technology Assessment. *New developments in biotechnology: Ownership of human tissues and cells*.

Office of Technology Assessment. (1987). *New developments in biotechnology: Public perceptions of biotechnology*. Washington, DC: U.S. Government Printing Office.

Halvorson, H.D., et al. (1985). *Engineered organisms in the environment: Scientific issues*. Washington, DC: American Society for Microbiology.

Risk/Benefit Analysis

Olson, S. (1986). Biotechnology: *An industry comes of age*. Washington, DC: National Academy Press.

Price, H.S. (1985). Biotechnology: The need for public education. *BioScience*, *35*(4), 211.

Pines, M. (1987). *Mapping the human genome*. Bethesda, MD: Howard Hughes Medical Institute.

Tiedie, J.M., Colwell, R.K., Grossman, Y.L., Hodson, R.E., Lenski, R.E., Mack, R.N. & Regal, P.J. The planned introduction of genetically engineered organisms: Ecological considerations and recommendations. *Ecology*, *70*(2).

Risk/Benefit Analysis

STUDENT PAGE 1

Risk/Benefit Analysis Sheet

Problem: _____

_____

_____

Facts About the Issue:

1. _____
2. _____
3. _____
4. _____
5. _____

Opinions About the Issue:

1. _____
2. _____
3. _____
4. _____
5. _____

Risks	Benefits
1. _____	_____
2. _____	_____
3. _____	_____
4. _____	_____
5. _____	_____
6. _____	_____
7. _____	_____
8. _____	_____
9. _____	_____
10. _____	_____

Decision/Supporting Statements _____

_____

_____

_____

_____

_____

Risk/Benefit Analysis

STUDENT PAGE 2

Scenarios

(Scenarios 5-10: From Myra I. Roche, M.S. and Barbara Wedehase, A.C.S.W., Genetic Counseling Program, University of North Carolina.)

1. Thomas and Lois, a married couple, were thrilled when Lois found out that she was pregnant. The couple had tried for three years to have a child. Twelve weeks later, after Lois had made her 3rd visit to her obstetrician, their joy turned to sorrow. Amniocentesis had revealed that their child is carrying the gene for cystic fibrosis (CF). What options are available to the parents?

2. James, a recent graduate from a mid-western teachers' college was excited about his new job as a biology teacher. However, one week prior to the opening of school, James was notified by the school board that he would not be offered employment due to the fact that he tested positive for the existence of a gene for Huntington's Disease. According to the school board chairperson, their decision was based on the fact that James would eventually cost the school system hundreds of thousands of dollars in health benefits. Should James' individual rights be protected? Should he be given employment? Did the school board act within the boundaries of law?

3. Bob, a 40-year-old, self-employed accountant, has a wife and three children, aged 8, 6 and 2 years old. Bob is currently involved in a lawsuit with a life insurance company. After the birth of his third child, Bob was refused life insurance based on genetic screening that the insurance company required as a condition for filing the policy. The genetic test showed that Bob is carrying a dominant gene for Familial Alzheimer's. Does the insurance company have the right to require the test for a condition to filing life insurance? Did the insurance company indeed have the right to refuse Bob's application for life insurance?

4. Carl and Nancy obtained a visa to enter the U.S. several years ago and are now applying for citizenship. The U.S. Department of Immigration and Naturalization has informed Carl and Nancy that they would be denied U.S. citizenship based on a genetic screening test that was performed as a condition for citizenship. What rights should governments have to base citizenship evaluation on genetic factors?

5. Recently a couple conceived a child in hopes the baby's bone marrow cells could save the life of a teenage daughter who is dying of leukemia. Is it right to conceive one person to serve the needs of another? Can parents distribute one child's bodily parts to save another child? Does one have a right to "grow-his-own-spare parts"?

Risk/Benefit Analysis

STUDENT PAGE 3

More Scenarios

6. You are the parents of a newborn who has just been diagnosed as having cystic fibrosis (CF) by newborn screening. You have been told that the test in mandated by the state, that there are five false positives for every true positive and that re-testing cannot be done until next month because of the volume of babies needing to be re-tested. What are your reactions regarding the advantages and disadvantages of newborn screening for CF?

7. Your six-year-old son has just been diagnosed as having Duchenne Muscular Dystrophy (DMD). You have spent two years and a large amount of money going from expert to expert to try to determine the cause of his muscle weakness. You have an 18-month-old son at home and your wife is pregnant, past the point of options regarding prenatal diagnosis. You discover that babies in a neighboring state are routinely screened at birth for DMD. What are your reactions regarding the advantages and disadvantages of newborn screening for DMD?

8. A fireman has a positive pre-symptomatic test for Huntington's Disease (HD). He has not told his employer because he fears he will be fired. He has only a few more years to go before he can retire comfortably with 20 years on the force. Meanwhile, he worries that he is already in the early stages of the disease, because he is starting to make errors in judgment. The last time he went out to a fire, the chief told him to pull the truck forward. Instead he put it in reverse.

9. The father of two young adults is in the early stages of Huntington's disease (HD), but is still mentally competent. Both his children want pre-symptomatic testing. He is willing to permit the use of his DNA so that his daughter can be tested, but refuses to permit its use for his son. He believes the boy is too emotionally unstable to cope with bad news. Can he resist the use of his DNA?

10. Mr. Hodges has Huntington's Disease (HD). Since he has been unable to work for the last three years, he, his wife and their three teenage children have been on government-assisted welfare programs for more than two years. As his disease progresses, Mr. Hodges is very likely to be institutionalized in the near future. It is estimated that the Hodges family will cost the government $150,000. Because society must take responsibility for the Hodges family, should screening be compulsory for the children? If they are tested, would you require full disclosure of all information but no further government interference or should prenatal testing be mandatory when the teenagers become pregnant? Would you favor discontinuing government aid if affected individuals chose to have children even after they have received complete information regarding the potential genetic risks?

Risk/Benefit Analysis

STUDENT PAGE 4

More Scenarios

11. A man, age 24, at risk for Huntington's disease (HD), does not wish to know whether he has the gene. Nevertheless, he does not wish to pass it on to his children. He and his wife request non-disclosing prenatal diagnosis that will tell them either that the fetus does not have HD or that it has the same 50% risk as its father. He says if the fetus has a 50% risk, they will terminate the pregnancy. His wife is not in full agreement with him, however. If she carries an at-risk fetus to term, and if the father subsequently develops HD, then the child will know (with 98% accuracy) that she/he also carries the HD gene. In effect, the child will have been tested without giving consent.

12. You have inherited a plot of land in California that seems to be ideal for a pick-your-own strawberries business. However, the early blossoming plants are often threatened by late frosts and records show that farmers in the area have lost money over the last five years. You are faced with using current frost protection – wind machines, water spraying, or smudgepots – or using the genetically engineered Ice-minus bacteria. What are your responsibilities regarding your business and protection of the environment?

13. Mr. McGregor has a small farm that is bounded on the north by a large truck farm that grows peanuts, potatoes, broccoli, tomatoes and celery. A corn and grain farm bounds McGregor on the south. McGregor is an organic farmer with one major pest, the rabbit. The owner of the truck farm applies insecticides very heavily and the grain farmer uses herbicides annually. What human problems could result from the practices on these three farms?

14. Dietary intake of potential carcinogens and teratogens has been of great public concern recently. Based on the Environmental Protection Agency's human "reference dose" and evidence available, compare TCDD (dioxin) with alcohol and broccoli. Which one(s) pose the greatest human dangers?

The Ice-Minus Case

Ability level .....Grades 7-12
Class periods required .....Approximately 5. If necessary, modify to fit your needs

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Introduction

In this lesson students assume the roles of individuals in a community struggling with the dilemma of whether to allow the release of genetically engineered bacteria. The use of simulations in the classroom is a good way for students to learn content *and* develop effective communication skills. It is also an excellent way for them to develop socialization and collaborative learning skills. The students become immersed in their roles, playing them as if they actually are the individuals they portray.

Objectives

After this activity students should be able to:

1. Explain the process of DNA recombination.
2. Discuss some of the controversy behind the release of genetically altered organisms.
3. Understand the way that science affects society and visa versa.
4. Formulate and present a coherent argument in a debate.

Materials

- overhead projector
- folders for each student containing:
  - their role description
  - the listing of "References for Characters"
  - the Risk/Benefit Analysis sheet
  - a photocopy of the referendum ballot
  - photocopies of Student Pages from this lesson

Teacher Preparation

1. Carefully read through the Teacher's Script and Student Pages.
2. Determine how you will assign roles. Keep in mind the following:
   - Every student must have a role. (You may create new roles or see how different students handle the same role with a name change.)
   - Mayor Perry, Darwin, Mendel, Hershey/Chase and Cohen should go to the most responsible students.
   - The gender of the student need not match the gender of the role.
3. Prepare the student folders. You can either give students the detailed role descriptions or let them have the summaries and flesh out the details on their own.
4. Schedule one to two class periods for library research time.

Teaching Tips

1. It is important to remember that the teacher is **not** an active participant in simulations. The students are the participants. You should limit their role to coordinating the simulation's flow and advising students how to research and present their character.
2. The Mayor and neutral roles require more help than the other roles – teachers may serve as "assistant to the mayor" or "lab assistant" to these characters and give clarifying explanations when needed.
3. When students are not testifying, they act as council members or voting members of the town. Reinforce to your students the idea that they will be required to vote and justify why they voted a certain way on the issue.
4. There are many different ways to assess student performance:
   a. a class participation grade
   b. evaluation of the role presentation
   c. a comprehensive test of the content presented during the simulation
   d. a student essay that defends his or her voting position
5. You may want to videotape the simulation for use in future classes.

Procedure

I. *Introductory class discussion*

1. Explain (or ask students to explain) the connection between genes, proteins and characteristics that organisms possess.
   Discuss what crossbreeding is and how it results in new genetic combinations. Explain that Recombinant DNA techniques also result in new genetic combinations.
   Pose the following questions:
   What are the advantages and disadvantages of crossbreeding?
   Is rDNA technology an extension of cross-breeding techniques?
2. Tell students that during this activity they will investigate a real example of gene alteration in which bacteria are used to prevent frost damage on plants. The ice minus case is an historical event that occurred in California and upon which our simulation is modeled.

Adapted from lesson "Biotown" in *BIOTECHNOLOGY: Classroom Activities, Labs, and Experiments.* (1984). Teachers Honors Workshops on Biotechnology, National Science Teachers Association.

The Ice-Minus Case

3. Hand out the student folders and pages and explain the procedure.
4. Explain any constraints the students may be under such as time limitations for presentation, use of props, accuracy of information presented (can they make up information, or must it be based strictly on facts?), etc.

II. *Library Research*

Students can use the "References for Characters" pages in their folders to help them research their role.
Be careful not to give too much help. Guide them to read the articles for facts and opinions that support logical arguments for their character's view point.

III. *Simulation of Town Meeting*

1. Follow the Teacher's Script
2. Collect written descriptions and outlines from each student after their testimony.

IV. *Referendum*

1. Collect student ballots and announce decision.

Resources

Jones, K. (1980). *Simulations: A handbook for teachers.* London: Kogan Page.

Jones, K. (1985). *Designing your own simulations.* London: Methuen.

Hollinshead, B. & Yorke, M. (1981). *Perspectives on academic gaming & simulation 6. Simulation and games: The real and the ideal.* London: Kogan Page.

Milroy, E. (1982). *A practical guide.* Aberdeen, Scotland: Aberdeen University Press.

Thiagrarajan, S. & Stolovich, H.D. (1978). Englewood Cliffs, NJ: Educational Technology Publications.

Tyson, R. (1986, May 12). Critical decisions due today. *USA Today.*

Introduction of recombinant DNA. Engineered organisms into the environment: Key issues. (1987). Washington, DC: National Academy Press.

Teacher's Script for the Ice Minus Case

Day 1

Teacher:

"In order to find facts and weigh opinions about environmental release of genetically altered organisms, we will become concerned citizens in our own town of _____ (Make up a name or use your actual town or community name). A genetic engineering company wants to field test its bacteria near our community at Alexander's Strawberry Farm. We will have a town meeting to decide if we will allow it. Some of us will play townspeople, and some of us will play the roles of outsiders who are called in to testify. Our town has a unique advantage: We can call history's major biologists back from the dead to get their advice. Some of us will be those biologists. Everyone will have a role. After testimony, we will act as our town council and vote."

Set a serious tone and briefly explain the sequence of today's events:

"The Mayor will convene the meeting and invite Dr. Cooper to propose his field test and development plans. The Mayor will pose questions that deal with the scientific background that he feels the town council needs in order to consider Dr. Cooper's proposal. These questions will be addressed by pivotal scientists from the past."

Advise the town council to take notes.

Turn the council meeting over to Mayor Perry.

Mayor Perry will convene the meeting and invite Dr. Cooper to propose his field test and development plans. Dr. Cooper will take three to five minutes.

Mayor Perry states that before we can question Dr. Cooper or hear more testimony, we need to have some scientific background on a few specific questions. He questions, in order, Darwin, Mendel, Hershey and Chase. Each will take five to ten minutes to answer.

If the Hershey/Chase and Cohen characters struggle with their explanations, they might call on their lab assistant (teacher) for help.

Darwin will explain the following statement and questions: The diversity of species on earth stems from natural selection. What is natural selection and how did you determine it existed? What effect might rDNA technology have on natural selection?

Mendel will answer these questions: Could we not just breed frost resistant strawberries? Also, could the genetically altered bacteria cross with wild bacteria and produce a dangerous hybrid?

Hershey and Chase will answer the statement and questions: There are many different molecules in cells. How did you find out which molecule controls heredity? Will changing DNA change heredity? *(Hershey and Chase may take a little longer than 10 minutes and need some help with the answer.)*

Teacher:

Summarize Darwin's, Mendel's, Hershey's and Chase's statements. Ask the students to share ideas about what they heard that is significant to the problem. If students hesitate, the teacher should initiate some questions.

Cohen will answer the statement and questions: How can you actually take a gene from one organism, put it in another one, and get the gene to work? Cohen will briefly describe his work and describe how restriction enzymes work.
He will demonstrate how DNA is cut and pasted to produce rDNA. *(You may suggest to Dr. Cohen that he use a "dry lab" activity to simulate the cutting and*

The Ice-Minus Case

recombining of plasmid DNA with restriction enzymes. Another alternative is to use transparencies to illustrate the processes. Advanced students can do this independently as homework.)

Teacher:

Review Cohen's work and tie it to Mayor Perry's question: How can you actually take a gene from one organism, put it in another one, and get the gene to work? Cohen's work added a new gene to a bacterium. Get students to speculate how a similar procedure might be used to remove a gene, as Dr. Cooper's company did.

Dr. Cohen will explain how bacteria is prepared to incorporate a gene. Before Dr. Cohen could get his recombinant DNA into the bacteria, he had to make the bacteria "competent" to take up DNA.

Teacher:

Explain the unproven theory of what the competent cell procedure does to the cell membrane to bring the DNA into the cell (zones of adhesion, etc.), depending on the level of the students. Illustrate the relationship between Dr. Cohen's explanation and the town's impending decision about field testing the bacteria. We're looking at what Dr. Cooper has done to the bacteria he wants to spray in our fields. His/her company has taken out a gene instead of adding a gene as Cohen did, but the procedure for getting the altered gene into the bacteria is the same. Plasmid DNA was isolated from *Pseudomonas*, a gene was removed, and the cells were made competent to take up the altered DNA. Bacterial transformation occurred and the bacteria changed phenotypically.

Remind students that tomorrow they will make their presentations.
Because of the time limit, citizens must be very concise, and tomorrow's speakers might want to polish what they have to say. It is important to maintain a brisk pace in this activity.

Day 2

Mayor Perry will call on Dr. Cooper to restate his proposal to field test bacteria and locate research and production facilities in the town.

Teacher:

Briefly review statements and answers to questions from Darwin, Mendel, Hershey/Chase and Cohen (4 minutes).

Describe the remainder of the council meeting as an opportunity to hear facts and opinions for and against the field test. Remind citizens to try to separate facts and opinions. Weigh each in light of what they have learned about recombinant DNA organisms and take notes.
Ask the students to make two lists, one of facts and one of opinions.

Mayor Perry calls pro and con testimony.
Each presentation lasts three minutes or less. The mayor should give speakers a 30-second warning.

Ideally, hear several of one kind, and then several of the other. Grouping all of one kind of testimony together prejudices listeners in favor of the last kind heard. Alternating every time tends to obscure the arguments.

If a student's presentation is especially unclear, help them restate it when they finish. If the student playing Mayor Perry is capable of making such a restatement accurately and kindly, this would be preferable.

When the presentations are complete, Mayor Perry will open the floor for questions. All speakers, including Dr. Cooper, Darwin, Mendel, Hershey / Chase and Cohen are available for questions. Any citizen, including the teacher, may ask questions of any presenter. Questions and answers should be brief, to-the-point and polite. Students should stay "in character" as they answer questions. Remind the citizens to look for logical inconsistencies and incorrect information in what others say. Advise them to use their notes.

Students should take notes on questions and answers.

Day 3

Teacher:

Begin a Risk/Benefit Analysis of the issue. Students should use the photocopied form in their folder. Have the students share facts that have surfaced during the town council meeting concerning the field test of the ice-minus bacteria. Guide the citizens in weeding out opinions.

Remind students of any major points they might have forgotten. Discuss cost the effectiveness of other frost protection methods such as wind machines, smudge pots and water spraying.

Mayor Perry will convene the meeting and state that testimony is complete. The town will then decide if it will allow the field test. Students should **not** vote the way their character would. Instead, ask students to vote as an ordinary citizen if such a field test were to be conducted near their town.

Teacher:

Use the ballots in the student folders for voting.

Collect ballots, Risk/Benefit sheets, notes, description of character, etc.

The Ice-Minus Case

STUDENT PAGE 1

Background Reading

The Ice-Minus Story

Pseudomonas syringae (Soo-doe-moan-us sir-in-gay) is a common bacterium found on plants. The cell membrane of *Psueudomonas* contains a protein that promotes ice crystal formation on the leaves and stems of plants. Ice crystals rupture the cell membranes, and *Pseudomonas* cells feed on the damaged plant cells. This form of *Pseudomonas* is sometimes called the "Ice-plus" variety (or Wild type) because it contains the ice-promoting gene.

Scientists discovered that when the protein that promotes ice crystal formation on *Pseudomonas* is absent, frost crystals do not form as readily. Using restriction enzymes, scientists can remove the gene in the Ice-plus form of *Pseudomonas*. This "Ice-minus" form is exactly like the Ice-plus form except for the one deleted gene. Plants that are sprayed with Ice-minus bacteria do not freeze until the temperature goes below 27°F. The potential for reducing agricultural losses due to frost damage is enormous.

Ice-plus and Ice-minus *Psuedomonas* both occur naturally. The Ice-plus is the dominant form. What do you think is the selective advantage of the Ice-plus form? Would it be possible to spray plants with enough of the ice minus form to replace the Ice-plus form of *Pseudomonas*? Laboratory tests were successful, but scientists needed to test their hypothesis outdoors.

In California, Advanced Genetic Systems applied for permission to field test their Ice-minus bacteria outdoors. The people who lived near the test site had to decide if the altered bacteria might harm them or the environment.

The Ice-Minus Case

STUDENT PAGE 2

General Instructions

Library Research

The more thoroughly you research your role, the better your presentation will be.

1. Use a minimum of two articles.
2. Write a one-page description of your character's position based on information from your research.
3. Outline the speech you will deliver during the meeting.
   (The one-page description and the speech outline will be due after your character speaks.)

Testimony

Your Own

A. When you present your testimony you should speak in the first person and take on the personality of that character.

B. When appropriate, use visuals to improve your presentation.

Others

A. Consider the following questions as you listen to testimony:
   - Is it dangerous to use rDNA techniques to alter genes in an organism?
   - Are genetically engineered organisms and naturally occurring organisms with the same traits identical?
   - Will rDNA techniques create new pests or organisms that cause disease?
   - Will rDNA techniques create new organisms that cannot be controlled once they are released into the environment?

B. In hearing testimony, council members should look for:
   1. logical inconsistencies.
   2. incorrect information
   3. what isn't being presented – what are the unasked questions?

The Town M eting

Day 1

1. The company manufacturing the altered bacteria will present its proposal to locate in our town, bringing new employment and tax money, and to field test the bacteria in our fields. *Company representatives will have 10 minutes to speak.*
2. Scientists will present neutral testimony designed to provide background and clarify facts. *Each scientist will have 10 minutes to speak.*

Day 2

1. Pro and Con view points will be presented. *Each speaker will have three minutes.*

Day 3

Finally, the class will vote as to whether or not they believe the bacteria should be released.

The Ice-Minus Case

STUDENT PAGE 3

Risk/Benefit Analysis Sheet

Problem: _____

_____

_____

Facts About the Issue:

1. _____
2. _____
3. _____
4. _____
5. _____

Opinions About the Issue:

1. _____
2. _____
3. _____
4. _____
5. _____

	Risks	Benefits
1.	_____	_____
2.	_____	_____
3.	_____	_____
4.	_____	_____
5.	_____	_____
6.	_____	_____
7.	_____	_____
8.	_____	_____
9.	_____	_____
10.	_____	_____

Decision/Supporting Statements _____

_____

_____

_____

_____

The Ice-Minus Case

STUDENT PAGE 4

Field Test Referendum Ballot

Check the appropriate box and write a brief paragraph stating the reason for your vote.

[ ] I am in favor of allowing the field test of Ice-minus bacteria near our town.

[ ] I oppose the field test of Ice-minus bacteria near our town.

The reason for my vote is: _____

_____

_____

_____

(Use back if necessary.)

Field Test Referendum Ballot

Check the appropriate box and write a brief paragraph stating the reason for your vote.

[ ] I am in favor of allowing the field test of Ice-minus bacteria near our town.

[ ] I oppose the field test of Ice-minus bacteria near our town.

The reason for my vote is: _____

_____

_____

_____

(Use back if necessary.)

Mayor Perry

You have seen newscasts about Ice-minus and read newspaper reports. You heard Dr. Cooper's proposal but you cannot decide whether to allow him to conduct a field test and build his research and production facilities in your town. You think the town council should decide, and that all sides of the case should be heard. The town stands to gain in taxes and employment if Dr. Cooper's company locates here. But some people say there are too many unknowns in what Dr. Cooper's company wants to do. You have called the town council together for a meeting to decide whether the town should allow the field test. They will decide yes or no.

Your biggest job in this meeting will be in keeping speakers to the point. When they get off the point, ask them questions about the town's specific problem. Make them relate their talks to the question: "Should we or shouldn't we allow this field test?"

Dr. Cooper and the neutral speakers, who speak on the first day of testimony, may stray a bit further than the others because they are giving us background.

During the Town Meeting

Call Dr. Cooper to propose his field test to the town. Ask him to explain what he intends to do and how the bacteria he has made are different from the ordinary bacteria now living in the fields. Ask him if he thinks there is any conceivable danger. Ask him to explain the benefits the town might gain from his locating here.

Call neutral speakers. Each one gets 10 minutes to speak. Hershey and Chase may take a little longer. Give all speakers a one-minute warning when their time is almost up. Call the speakers in the following order, and ask them the following questions:

Mr. Charles Robert Darwin

1. What contribution did you make to modern genetics?
2. Can your theory of natural selection shed some light on whether the altered bacteria can overcome the Wild type? What will be the consequences if they do?
   • What might happens if we overload the environment with one kind of bacteria?

Father Gregor Mendel

1. What contribution did you make to modern genetics?
2. Might the traits of the Ice-minus bacteria be passed to other bacteria, and might this cause problems?
3. Why can't we just develop hybrid plants that resist the frost on their own?

Dr. Alfred Hershey & Martha Chase

1. What contribution did you make to modern genetics?
2. How do we know that DNA is all we're concerned with here? Couldn't there be something else inside of the bacteria that controls their traits?
3. Just because Dr. Cooper has recombined DNA, does that mean he knows exactly what his bacteria will do?

Dr. Stanley Cohen

1. What contribution did you make to modern genetics?
2. What has Dr. Cooper done to the DNA of the wild bacteria to make it special?
3. Is it inherently dangerous to use rDNA techniques to move genes between unrelated organisms?
4. How can you actually take a gene from one organism, put it in another one, and get the gene to work?

Dr. Cohen will take more time to make his explanations.

After Dr. Cohen has finishedhis/her discussion, begin to call witnesses. Say, "Today we will hear facts and opinions for and against the field test. Try to separate facts from opinions. Take notes for a question and answer session after testimony is complete. Make a list of facts and a list of opinions as you listen. Do not interrupt speakers. Each speaker has three minutes, and I will give a 30-second warning."

The Ice-Minus Case

STUDENT PAGE 6

Call witnesses in groups of two or three against the field test, then two or three for the field test. Each person has three minutes to speak. As soon as one is finished, call on the next one. If someone gives an unclear presentation, in as nice a way as possible, help them clarify what they have said. Keep things moving.

After the last witness has spoken, open the floor for questions. Keep questions and answers brief and polite, as they are in real town meetings. At the end of discussion everyone will begin work on Risk/Benefit Analysis.

After the Risk/Benefit Analysis is complete and council members have had time to consider, tell the citizens that from this point on we will not act out characters, but act as concerned citizens. Call for a vote. The class will finish their risk/benefit sheets. If there is time, have a brief discussion. Hand out the ballots for voting. Explain that ballots will be collected and tallied and that the results of the vote will be announced tomorrow.

Charles Darwin

You are a famous British naturalist who sailed around the world in the late 1850s on the ship HMS Beagle. As the ship sailed past South America, it stopped for awhile at the Galapagos Islands off the coast of Chile. These islands are far from land, so the animals on them developed independently. On the islands, you saw diverse animal life, each animal adapted to its own specific habitat. The islands were for you a special isolated laboratory where you asked your question: "How do you account for the diversity of the species?" In asking this question, you founded modern genetics and set the stage for the molecular biology that was to come nearly a century later.

As a well-trained observer, you noticed differences between the animals on the island and those you saw in other parts of the world. You noticed, for example, that some of the same kinds of birds lived on both the Galapagos and the mainland. The beaks of the Galapagos variety are short and wide, while the beaks of this variety on the mainland are long and slender. You noticed that the birds with the short, sharp bills are birds that must break open seeds.

You thought about these differences. Finally you guessed that the animals with modified features were survivors of this harsh environment. You investigated more animals to try to gather evidence to support your hypothesis. You determined that the differences you found were all related in some way to how the birds live. For example, birds that lived around water had webbed feet, and birds that ate insects from the bark of trees had long, slender beaks.

You thought that animals with traits not suited to the environment die off before they can reproduce. Animals with traits that help them cope with their environment are more likely to survive and reproduce. These animals pass their adapted traits on to the next generation. Gradually, by this natural selection, the entire species of animals changes. In 1859, you wrote *The Origin of Species* explaining how animals develop traits. You believe that all living things slowly evolve from pre-existing forms.

During the Town Meeting:

You and Mendel are the co-founders of modern genetics, so you are just the person to tell the town how the altered bacteria Dr. Cooper proposes to test might fit in to the environment. Although you know nothing of recombinant DNA, you can speak to questions of "natural selection" or "survival of the fittest." Ask your teacher for help in understanding Darwin's work as it relates to the problem. You'll have 10 minutes to briefly summarize the following. As you research the role, prepare to:

- Explain about the origin of species and the diversity among animals.
- Explain, in general, what kind of qualities make one organism more competitive than another? Explain the term "selection pressure" (ask your teacher).
- Competitiveness stems from an organism's relationship to certain factors of the environment. What are these factors? What happens when we overload the environment with one kind of bacteria, altered or not? Explain how using Ice-minus actually works in terms of "crowding-out" the Wild type.
- List three important questions to ask about releasing any new organism.

References

Conkin, P.K. (1989). Darwin revisited. *Kayes Historical Journal, 3*(6), 9-12.

Gregor Mendel

You are the famous monk, Father Gregor Mendel. You were the first person to relate the outward appearance of an organism (its phenotype) to its inner genetic composition (genotype).

You wondered how traits were passed on, and you experimented with garden peas to try to find out. You noticed that when pollen from tall purebred peas fertilized ovaries of short purebred peas, the offspring were all tall. When this second generation of all tall plants reproduced, some of the offspring were tall and some were short, even though the parents were all tall. In similar experiments with other traits, you got similar results. You guessed that each trait is determined by a pair of factors, one from each parent.

You determined that there is a basic unit of heredity, although you never found exactly what that unit was. You showed that traits are somehow passed from one generation to the next through this "basic unit of heredity."

You set down some basic laws: For instance, a parent's contribution to a trait is either "dominant" or "recessive." When body cells have two dominant genes or a dominant and a recessive gene, that plant or animal will look like the dominant characteristic. A cell must receive two recessive genes in order for the recessive trait to be seen when looking at the plant.

Finally, you wrote a paper called "Experiments in Plant Hybridization." Your paper described the rules for inheriting traits. With Darwin, you are considered by many to be one of the co-founders of modern genetics. Although you know nothing about recombinant DNA, and you did not study bacteria, you can speak to questions about the passage of traits in general.

During the Town Meeting

You'll have 10 minutes to briefly summarize the following:

- Briefly explain Mendel's Laws.
- The Ice-minus bacteria have a plasmid with a deleted gene. Bacteria share plasmids. What would happen if an Ice-minus shared plasmids with a Wild type bacteria? Would the Wild type become an Ice-minus type? Explain why not. How is a plasmid with a deleted gene like a recessive trait?
- The Ice-minus bacteria apparently do not make people sick but, if they were pathogenic, might disease-causing traits on the plasmids leak out into the environment and spread to other bacteria?
- Why isn't frost-resistance a trait in strawberry plants? Why can't we develop hybrid strawberries that resist frost?

References

Brannigan, A. (1979). The reification of Mendel. *Social Studies of Science* (Great Britain), *9*(4). 423-454.

Huxley, A.F. (1982). Centenaries. *Transactions of the Royal Society of Canada*, *20*, 27-34.

Van der Waerden, B.L. (1968). Mendel's experiments. *Centaurus* (Denmark), *12*(4), 275-288.

The Ice-Minus Case
STUDENT PAGE 9

Alfred Hershey & Martha Chase

Hershey and Chase should present this information together. (They developed the "Blender Experiment" together.) Divide the information appropriately in your presentation.

Hershey

(Department of Genetics, Carnegie Institution)

You were a geneticist in the late 1940s, working right after World War II. Mendel's work was rediscovered by Hugo de Vries and Carl Correns in 1900, and it showed the mechanism of evolution. After that, there was a lot of research in genetics. Each new discovery added to the knowledge about chromosomes and genes. Scientists used the discoveries of those before them, and added new knowledge based on their own research.

Your research was greatly influenced by the following four major discoveries in genetics:

• In 1902, Walter Sutton asked, "Where is the genetic information located in the cell?" He presented the chromosomal theory of heredity that says that genes are actually located on chromosomes.

• In 1928, Frederick Griffiths observed a change in physical form of bacterium that was brought about by contact with another bacterium. He determined that genetic information could be transferred from one bacterium to another, and began to ask the question, "What 'principle' transfers genetic information?"

• In 1940, George Beadle and Edward Tatum asked, "What do genes really do?" They showed that each gene controls the production of one specific protein.

• Finally, in 1944, Oswald T. Avery and his associates carried on Griffith's quest for the "transforming principle" and showed that DNA is the molecule that carries genetic information. Unfortunately, their work wasn't well accepted.

Chase

(Cold Spring Harbor Laboratories, New York)

You and Alfred Hershey wanted to do further research to prove that DNA alone carries genetic information for reproduction. Using the information from the above four discoveries, you used tiny viruses called bacteriophages (phages, for short) to investigate heredity. In your now-famous "blender experiment," you and Hershey conclusively proved Avery's notion that DNA is the molecule of heredity – DNA alone carries genetic instructions to produce new cells.

You knew that a bacterial parasite called a "phage" reproduces inside bacterial cells. The phage injects its contents into the bacterium and somehow these contents direct the bacterium to produce new phages. The bacterium then bursts to release a new generation of phages. You hypothesized that the genetic component of phages was DNA, not protein.

Phages are composed of only an outer capsule of protein and an inner core of DNA. You ran two parallel experiments with two groups of phages. In one experiment, the protein capsules had radioactive isotope labels attached to them. In the other, you radio-labeled the DNA. You were able to observe what happened during phage replication (reproduction) by following these radio-labeled through the reproductive process.

You infected cultures of bacteria with the radio-labeled phages. You carefully timed the experiment so that there was just enough time for the phages to attach and for replication to begin. Then you put the cultures in an electric blender to separate the protein capsules and the DNA (hence the name "blender experiment"). You found that all the radio-labeled protein remained entirely with the capsule part of the phage. Only radio-labeled DNA was found inside the bacterial cells. Because of the results of this experiment, you were able to prove that it is DNA alone that carries the genetic instructions for developing new phages within the bacterial cell.

The Ice-Minus Case

STUDENT PAGE10

During the Town Meeting

You can come up with your own outline of what Dr. Hershey and Ms. Chase would say, but this script will help you order your explanation of the famous Hershey/Chase blender experiment. Be sure that both Hershey and Chase share information.

"You people want to know if recombinant DNA organisms are precisely-enough engineered so you don't have to worry about accidental side-effects. You think maybe Dr. Cooper's bacteria will do something unexpected. You think maybe something else besides DNA causes changes. Well, I don't know exactly what Dr. Cooper's bacteria will do. Recombinant DNA came after my time. But I can tell you that DNA and only DNA is the molecule of heredity. Most of what you call biotechnology is possible because I showed that DNA is the molecule to look at if you want to find the information for heredity."

"I'll tell you how we figured out that DNA determines what organisms are like. Back in 1945, we'd narrowed the possibilities to two molecules: It was either DNA or protein. We know one of these substances carried genetic information from parent to offspring. At that time, protein was the popular choice. We proved it was DNA, though, and did it by using an electric blender! I'll tell you about that in a minute."

"For our experiment, we used organisms called phages. Phages shoot their insides into bacteria. The stuff they shoot in, all by itself, produces more phages within bacteria. The neat thing about phages is that they are made of only our two suspects – DNA and protein."

"Here's what we did." Refer to the diagrams on the next page with the circled numbers. Use the following dialogue with the diagrams (photocopy or make an overhead) to explain Hershey's experiment:

• *Point to the shaded capsule (#1, top row)*
"We 'labeled' or 'tagged' the protein capsule in this group of phages with a radioactive substance. This group of phages had radioactive proteins that we could easily trace."

• *Point to the shaded DNA (#1, bottom row)*
"We used another radioactive substance to 'tag' only the DNA in these phages, so this group had radioactive DNA."

• *Point to the #2 figures top and bottom*
"We let these two groups infect two separate batches of bacteria."

• *Point to the shaded protein capsules (#2 top)*
"If **protein** is passed to subsequent phages, then we should find radioactivity. . ."

• *Point to #4 top*
". . . here in the protein capsules of phages that are produced from the infected bacteria."

• *Point to the shaded DNA (#2 bottom)*
"If **DNA** is passed to subsequent phages, then we should find radioactivity. . ."

• *Point to #4 bottom*
". . . here in the DNA of phages that are produced from the infected bacteria."

• *Point to #3 top and bottom*
"After an incubation time, we used blenders to shake the phages loose from the bacteria. We wanted the insides of the phage to go into the bacteria, but we didn't want any phages left stuck to the outside. After we shook them off, we separated the phages from the bacteria. We had to be sure we were **only** dealing with the stuff the phages had put **in**side the bacteria. Then we waited for the bacteria to get sick and burst out with a new generation of phages. And what do you think we found?"

• *Point to #4 top*
"These daughter phages had no trace of radioactivity. The phages reproduced but no phage protein was in the bacteria. If phage protein had been there, we'd have seen radioactivity. Therefore, no protein is needed for the phages to reproduce. Could protein be the molecule of heredity?"

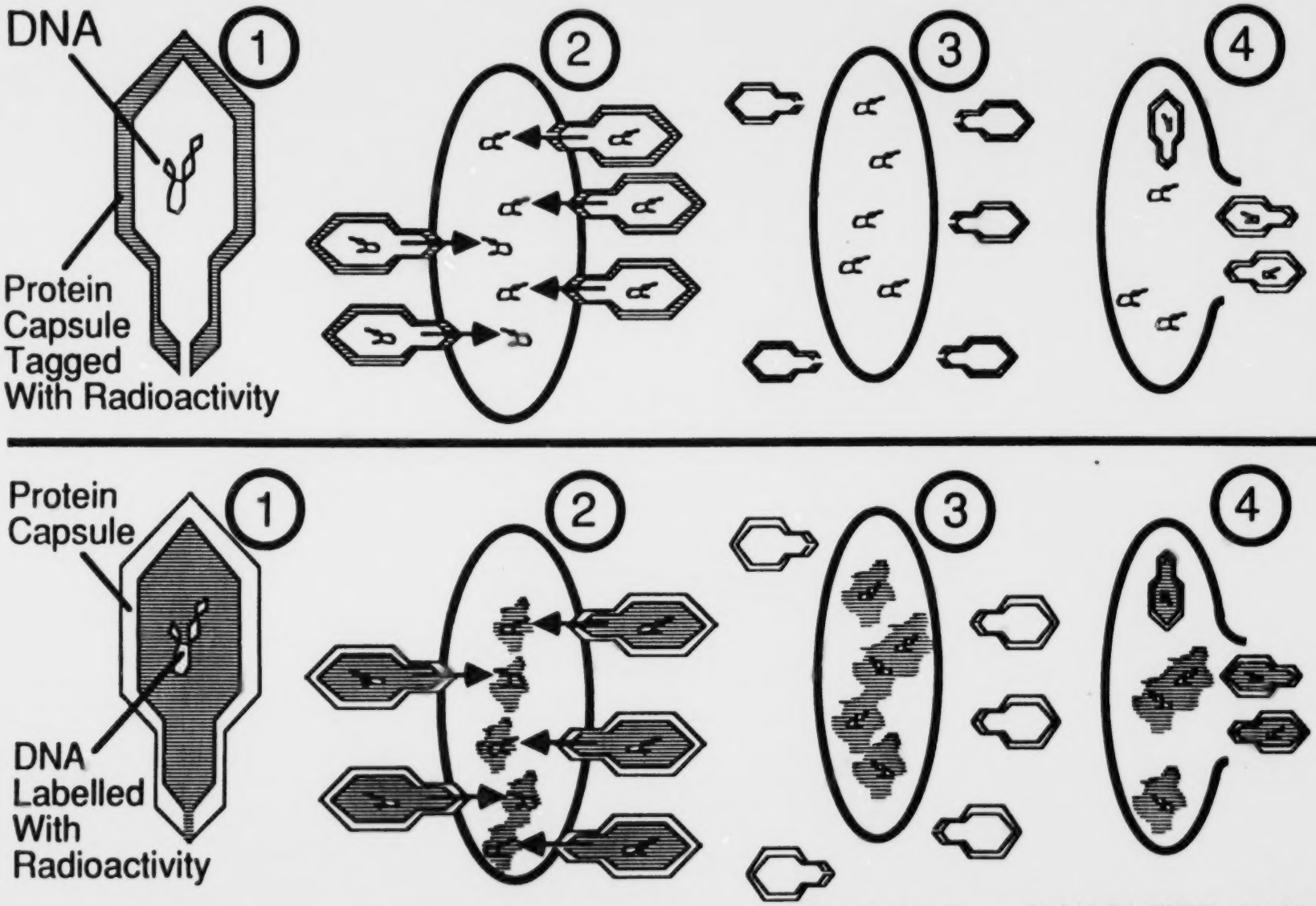
• *Point to #4 bottom*
"Down here was a different story. The bacteria were radioactive, and what's more, the daughter phages were radioactive. . ."

• *Point to #1 bottom*
". . . just like the parent phage back here. This proved that DNA was the molecule of heredity.

"You ask me if I'm sure DNA is really the principle of heredity. Yes, I'm sure. But as to what kinds of things could go wrong when you recombine it, I don't know. Maybe Dr. Cohen knows.

Hershey/Chase Blender Experiment

= RADIOACTIVE



The Ice-Minus Case

STUDENT PAGE 12

Stanley Cohen

You are a famous American geneticist whose work was influenced by others before you. In 1953, James Watson and Francis Crick synthesized the work of other researchers and made the famous spiral ladder model of the DNA molecule. Because of them, you know the structure of DNA. The rails of the ladder are alternating units of sugar and phosphate that run in opposite directions. Each rung is a pair of nucleotides connected by hydrogen bonds. The nucleotides are paired so that adenine always goes with thymine and cytosine always goes with guanine. Watson, Crick and other scientists had already shown how the DNA replicates during cell division and creates proteins through transcription and translation.

In 1972, the year before your experiment, Paul Berg used a new tool called "restriction enzymes" to cut DNA from several viruses. You carried Berg's work a step further. You isolated a small piece of DNA containing a gene for antibiotic resistance. Using a procedure similar to Berg's, you cut open a plasmid that had a gene for resistance to an antibiotic, and joined it to a plasmid with resistance to another antibiotic. In this way, you produced a recombinant plasmid with resistance to two types of antibiotics. You changed the genetic make-up of a living thing. You made recombinant DNA.

During the Town Meeting

You'll have 10 minutes to briefly summarize the following.

- Briefly describe (point out in the textbook, use a transparency, or draw on the board) the structure of DNA detailed by Watson and Crick, explain that it can be cut with restriction enzymes, then explain your experiment.
- Relate Ice-minus to recombinant DNA. What are scientists doing to the wild strain of the Ice bacteria to make it Ice-minus? Explain similarities to your experiment.
- Address the question: Is it inherently dangerous to use rDNA techniques to move genes between unrelated organisms?

Ideas

- When you finish your explanation, you or your lab assistant (your teacher) may demonstrate exactly how your experiment was done. You might recreate your experiment using a paper dry lab (ask your teacher about this).
- When the dry lab is over, explain that you have only shown the town council part of what recombinant DNA is all about. How do you get living bacteria to take up this plasmid? Explain how bacteria are prepared to incorporate a gene. Ask your lab assistant to explain competent cells and bacterial transformation.

References

Bearman, D. (1980). The twentieth century revolution in biology. *Isis*, *71*(256), 140-142.

The eighth day of creation: The makers of the revolution in biology. (1979). New York: Simon and Schuster.

White, B.N. & Holden, J.J.A. (1982). From genetic engineering to biotechnology. *Queens Q* (Canada), 89(3), 538-549.

The Ice-Minus Case
STUDENT PAGE 13

Brief Summary of Pro Characters:

Dr. Cooper
Ice-Minus Developer

The person proposing the field test to the town. Owner of Genetic Products, the company that plans to move to the town.

Mr./Ms. Davis
Professor of Microbiology

Thinks that this new technology has the potential for great benefits in medicine and agriculture. Feels safeguards already adequate.

Mr./Ms. Miller
State Industrial Biotechnology Association

Feels this experiment enhances the acceptance of the 18 statewide biotechnology firms.

Dr. Martin
Chairperson of the County Board of Supervisors

Feels that frost damage prevention in this area would be a tremendous benefit.

Mr./Ms. Fearrington
Biology Teacher

Wishes both students and parents were better informed on the issues.

Mr./Ms. Bynum
Owner of a Biotech Company Making Products in Another Field

Disagrees with the public fear that such organisms could become a nuisance.

Mr./Ms. Kahn
Construction Company Owner

Stands to benefit economically by the biotech company locating in town.

Dr. Mogumbi
Plant Pathologist

Believes the experiment does not pose a risk.

Mr./Ms. Schroeder
Farmer

Believes that Ice-minus will greatly help with the frost damage problem. It might save the farm.

The Ice-Minus Case

STUDENT PAGE 14

Dr. Cooper

Ice-minus Developer (Pro-Test)

A few years ago, you were a researcher with the local university. You and your colleagues developed mutant strains of bacteria, *Pseudomonas syringae*. You have since started your own company, Genetic Products.

The Wild type of bacteria that are the parents of the Ice-minus mutants have an ice nucleating protein on their surface. This protein helps ice crystals form on the surface of the plant's leaves and blossoms at 32°F. If this protein is absent, water will supercool on the surface of the leaves at about 27°F. Since it is the formation of ice that causes frost damage, the absence of the ice nucleating protein protects plants from frost as much as five degrees below the normal freezing point of water.

The Ice-minus mutant bacteria have the gene coding for the ice nucleating protein deleted. Thus, when these altered bacteria replace the wild type on the plant's leaves and blossoms, water can supercool rather than freeze and damage the plant.

Experiments in the lab have shown good results using *Pseudomonas syringae*, but the real test of its effectiveness will be seen in a field test where the bacteria will have to function in a real competitive environment.

You plan to test the bacteria on plants at the university field station, five miles outside of town. The field test involves putting Ice-minus mutants on the plants. Tests inside the laboratory have shown that the Wild type of bacteria and the Ice-minus derivatives compete for the same plant sites on a first-come-first-serve basis. The Ice-minus strain is slightly antagonistic to the wild type, so if you give it a head start, it can win out for awhile, and retard frost formation.

You have to do the field test of the Ice-minus bacteria in the Spring. Applications for permission to do the field test have gone to NIH, USDA and to EPA as well as this town.

You know that a similar bacteria is to be tested at about the same time by an industrial research group.

You would like to do your field test before the rival group, but doubt that it will be possible because private industry is not as restricted as you are.

If you are allowed to field test here, you intend to build your$20 million research and production facilities in town, bringing about 30 jobs and extra tax revenue to the area.

References

Rhein, R. Jr. (1985, January 14). Splicing together a regulatory body for biotechnology (need for federal guidelines in biotechnology industry). *Business Week, 69.*

Sharples, Frances E. (1987, March 13). Regulation of products from biotechnology (environmental applications and risk assessment). *Science, 235,* 1329.

Sun, M. (1987, August 21). Recombinant organisms pose no special hazard. *Science, 237* (1), 840.

Wessman, L. (1986, June 2). Davis Calgene will take its plant genes public (company profile). *Business Journal* Serving Greater Sacramento, *3*(1), 8.

Mr./Ms. Davis

Local Microbiology Professor
(Pro-Test)

You are a professor of microbiology. You have been involved in genetic engineering for several years. You have found genetic engineering research to be both harmless and beneficial. The experiments you know of were done using harmless organisms and produced beneficial products.

You have seen that this new technology has the potential for great benefits in medicine and agriculture. Medicines such as human growth hormone and insulin are now manufactured by bacteria, and you would like to see further developments such as these.

You realize that there must be strict precautions on lab research and field testing, but you feel that government restrictions provide adequate safeguards. More restrictions would tie the hands of scientists who are already careful. The track record has been good for this kind of research which had given increasing benefits to medicine, agriculture and industry in the last six years without even one harmful incident.

You really don't understand why the general public is so afraid of the new research. After all, these new organisms should be no more dangerous than the naturally occurring organisms from which they are derived. Actually, the genetically engineered organism would probably be at a disadvantage in the real world where it would be competing with naturally occurring strains – like hothouse plants competing with weeds.

Bacterial bits of genetic material are being transferred between species all the time. In labs the process is only accelerated.

You feel that the decision affecting the general public should not be made by people with little or no understanding of the process. You feel that special courts should be set up using judges with expertise in science.

You wish other townspeople understood the technology better. You were quoted as saying "Our politicians would have opposed the agricultural revolution if they had only been around at the time." You feel that politicians have done much damage by delaying the progress of beneficial research.

References

Battling biofundamentalists (includes article on American attitudes toward biotechnology). *Economist, 303*, 87.

Bugs in the system (risks and safeguards of releasing altered genes into the environment). (1986, June). *Scientific American, 254*, 62.

Chamberland, D. (1986, February 7). Genetic engineering: Promise & threat. *Christianity Today, 31*, 22.

Crawford, M. (1986, October 17). Regulatory tangle snarls agriculture research in the biotechnology arena. *Science , 234* , 275.

Culliton, Barbara J. (1985, October 25). Episcopal Church backs genetic engineering. *Science, 230*, 423.

Durbin, W.A. I. Jr. (1987, September 4). Should Christians oppose genetic engineering? *Christianity Today, 31*, 54.

End of the logjam in biotechnology (clear government regulations on genetic engineering). (1986, September 15). *Business Week*, p210.

Federoff, N. (1987, September 2). Impeding genetic engineering. *The New York Times*, 136, 21 (N), A27(L) col 1.

Fine-tuning biotech review, regulation. (1986, October 18). *Science News, 130*, 252.

Gabor, A. (1985, November 25). Fresh from the labs, a farming revolution. *U.S. News & World Report, 99*, 74.

Government regulations criticized (genetic engineering research hampered by regulations). (1986, June). *USA Today , 114* ,15.

Kleist, T. (1986, August 2). Biotech rules receive scrutiny. *Science News, 130*, 71.

Miller, J.A. (1984, March 24). The clergy ponder the new genetics. *Science News, 125*, 188.

Piller, C. (1986, October 25). From ice-nine to ice-minus; regulating altered genes. *Nation, 243*, 400.

The Ice-Minus Case

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Rogers, Michael. (1986, May 26). Tinkering with nature; Genetic engineering may revolutionize agriculture – but is it safe? *Newsweek, 107*, 54.

Sun, M. (1984, August 17). Biotechnology's regulatory tangle. *Science, 225*, 697.

Tangley, L. (1985, September). Releasing engineered organisms in the environment; assessing the risks of genetically engineered organisms will require closer collaboration between molecular biologists and ecologists. *Bioscience, 35*, 470.

Wortman, J. & Wenzel, L. (1986, January). Biotech regulatory plans move forward. *BioScience , 36*, 16.

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Mr./Ms. Miller

Director, State Industrial Biotechnology Association(Pro-Test)

You are the director of the SIBA, a biotechnology trade association. You see the whole Ice-minus issue as a landmark trial that will ease public concern about the hazards of releasing genetically engineered organisms into the environment. You feel this experiment enhances the acceptance of the 18 state biotechnology firms you represent.

You have the draft of the Environmental Impact Report and believe it conclusively shows that the nucleation-minus field test will have no adverse effects on the environment or human health.

You are concerned over the plans of the university to submit to yet another environmental assessment in response to the protests of the tests by politicians.

You know that the previous reports underwent exhaustive review by the federal government and every relevant federal agency – National Institutes of Health, Environmental Protection Agency, Department of Agriculture and numerous advisory boards of each of these organizations. A federal environmental assessment was conducted pursuant to the National Environmental Protection Act by the National Institutes of Health. At the conclusion of more than five years of assessment, no adverse environmental effect to the environment or people's health has been identified. A state permit wasn't even required after all of these assessments.

You believe that the assessment process was sufficiently long and expensive ($150,000), generating enough data to satisfy even the most critical opponent. You don't believe that further research will provide anything that has not already been shown. This repetition of effort will only redirect the attention of scientists who could move on to actual application of the successful processes – not to mention the extra cost involved in more research. You feel that delays such as those proposed by State Representative Hunt are bad for academic research and the biotechnology industry because the bad publicity and the economic burden may retard research.

References

Biotechnology gets good marks in survey. (1985, August 16). *Chemical & Engineering News, 63,* 16.

Crawford, M. (1987, June 5). Mixed views on biotech. (General public tends to favor genetic engineering experiments, but doesn't understand them). *Science, 236,* 1179.

Goldbaum, E. (1987, June 10). Poll results may bode well for biotechnology. *Chemical Week, 140,* 15.

Hanson, D. (1987, June 15). Survey finds public supports biotechnology. *Chemical & Engineering News , 65,* 22.

Sun, M. (1984, November 9). Committee vetoes proposal to end gene tests (National Institutes of Health. Recombinant DNA Advisory Committee). *Science, 226,* 674.

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Dr. Martin

Local Resident (Pro-Test)

You are the chairperson of the County Board of Supervisors. You have read the Environmental Impact Report and recognize the potential benefits which can accrue from the university's Ice-minus field test. If the results do show that the bacteria can prevent frost damage, this area, which is mostly agricultural, will have the protection it needs against the early spring frost. This is a common problem in this area which has suffered reduced crop productivity for the last two years.

Another tremendous benefit to the county is the proposed $20 million research/production facility that Genetic Products (who has been working with the university) plans to build if the field tests are successful. This will bolster the tax base and increase employment.

You realize the citizens of the county are lucky to have the university and to have the perfect climate for such an important research project, but you also have heard the concerns of the various citizens of the county. You also wonder what would happen if something did go wrong. The publicity alone may negatively affect the already slow economy of the area.

You want the research, but feel that the field test should be done in an alternative site more removed from this agricultural dependent area. If this is done, the county could escape unforeseen detrimental effects of the field test, yet reap the benefits if it succeeds.

You would like to see a public information campaign on their minimal adverse risks posed to the public's health and the environment from such experiments.

References

Antifrost bacteria: So far, so good. (1987, June 20). *Science News, 131,* 396.

Biotechnology gets good marks in survey. (1985, August 16). *Chemical & Engineering News, 63* , 16.

Biotechnology to change farm landscape. (1986, April 12). *Science News, 129,* 233.

Crawford, M. (1986, April 4). Larger public sector role sought on biotech. *Science, 232,* 15.

Crawford, M. (1987, June 5). Mixed views on biotech (general public tends to favor genetic engineering experiments, but doesn't understand them). *Science, 236,* 1179.

Genes, not jeans (biotechnology may change agricultural economics). (1988, May). *Scientific American, 254,* 66.

Genetic engineering is worth the risk. (1985, November 18). *Business Week,* p144.

Goldbaum, E. (1987, June 10). Poll results may bode well for biotechnology. *Chemical Week, 140,* 15.

Hanson, D. (1987, June 15). Survey finds public supports biotechnology. *Chemical & Engineering News, 65,* 22.

Miller, J.D. (1987, June). Scientifically illiterate (Research on adults' understanding of scientific terms and concepts). *American Demographics, 9,* 26.

Smithy, Emily T. (1987, February 23). Bacteria + biotech = heartier harvests. *Business Week,* p. 122.

Wessman, L. (1986, June 2). Davis' Calgene will take its plant genes public (company profile). *Business Journal* Serving Greater Sacramento, *30,* 8.

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Mr./Ms.Fearrington

Biology Teacher (Pro-Test)

You are in favor of the Ice-minus experiment. You understand the recombinant DNA technology and feel that the products from this research will be very beneficial.

You have heard the concerns of the parents through the comments of the children you teach. Just yesterday, someone asked if Ice-minus bacteria could take over the crops and kill them. A friend asked if the Ice-minus bacteria would prevent the freezer from making ice cubes. Some people think the field test will cause cancer.

You explained to your students that recombinant DNA techniques such as those in the Ice-minus experiment involve only a slight change in the genome of bacteria that normally live on the crops. The gene in the bacterium that allows ice crystals to grow has been removed. All other traits are the same. It will still behave just as the original and will have the same growth requirements as the original. The only thing different is that it will not let ice crystals grow on its surface.

You believe the parents have the responsibility to become educated on this issue. The biotechnology industry could help a great deal by providing public information to the citizens so they will be aware of the true benefits and risks.

It would surely make your job easier if you didn't have to undo the damage of bad information and lack of knowledge.

References

Biotechnology gets good marks in survey. (1985, August 16). *Chemical & Engineering News, 63*, 16.

Crawford, M. (1987, June 5). Mixed views on biotech (General public tends to favor genetic engineering experiments, but doesn't understand them). *Science, 236*, 1179.

Goldbaum, E. (1987, June 10). Poll results may bode well for biotechnology. *Chemical Week, 140*, 15.

Hanson, D. (1987, June 15). Survey finds public supports biotechnology. *Chemical & Engineering News, 65*, 22.

Miller, J.D. (1987, June). Scientifically illiterate (Research on adults' understanding of scientific terms and concepts). *American Demographics, 9*, 26.

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Mr./Ms. Bynum

Biotech Company Owner (Pro-Test)

You have been doing research altering *Rhizobia* bacteria to improve their nitrogen-fixing properties.

You are aware of the controversy over allowing field tests of altered bacteria and disagree with the public fear that such organisms could escape into the environment and become a nuisance. You think the public should understand that only small features of the microoganisms are being altered and that the effects of the change are predictable.

In your experience, you have noticed that the changes which were made were usually detrimental to the organism. You have had great difficulty getting *Rhizobia* mutants to survive after introduction into agricultural soils. You feel that Dr. Cooper's altered bacteria will experience the same limiting factors of the environment and will not escape and cause trouble. Even if that was possible, the Ice-minus experiment is taking away a characteristic of the bacterium rather than adding a new destructive one.

You are distressed over the red tape involved with approval of genetic engineering research and especially field tests because it tends to discourage further research in this area.

References

Brill, W.J. (1985, January 25). Safety concerns and genetic engineering in agriculture. *Science, f227,* 381.

Bugs in the system (risks and safeguards of releasing altered genes into the environment). (1986, June). *Scientific American, 254,* 62.

Crawford, M. (1986, October 17). Regulatory tangle snarls agriculture research in the biotechnology arena. *Science , 234 ,* 275.

End of the logjam in biotechnology (clear government regulations on genetic engineering). (1986, September 15). *Business Week,* p210.

Federoff, N. (1987, September 2). Impeding genetic engineering.*The New York Times,* 136, 21 (N), A27(L) col 1.

Fine-tuning biotech review, regulation. (1986, October 18). *Science News, 130,* 252.

Government regulations criticized (genetic engineering research hampered by regulations). (1986, June). *USA Today, 114,* 15.

Kleist, T. (1986, August 2). Biotech rules receive scrutiny. *Science News, 130,* 71.

Miller, Henry I. (1987, April 10). The case for qualifying "case by case" (genetically engineered organisms experiments evaluated on case by case basis). *Science, 236,* 133.

Miller, J.A. (1986, January 25). Fight continues over gene-splice release (field test by Advanced Genetic Science Inc.). *Science News, 129,* 56.

Rogers, Michael. (1986, May 26). Tinkering with nature; genetic engineering may revolutionize agriculture – but is it safe? *Newsweek, 107,* 54.

Sun, M. (1984, August, 17). Biotechnology's regulatory tangle. *Science, 225,* 697.

Sun, M. (1987, August 21). Recombinant organisms pose no special hazard. *Science, 237 ,* 840.

Tangley, L. (1985, September). Releasing engineered organisms in the environment; assessing the risks of genetically engineered organisms will require closer collaboration between molecular biologists and ecologists. *Bioscience, 35,* 470.

Wortman, J. & Wenzel, L. (1986, January). Biotech regulatory plans move forward. *BioScience , 36,* 16.

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Mr./Ms. Kahn

County Resident (Pro-Test)

You are the owner of a construction company. Business has been slow for the past two years due to the depressed economy of this mostly agricultural area.

You have heard about the Ice-minus field test and think it may result in some very beneficial effects on the community. If the experiment works, the farmers may be able to extend the growing season and that could help everyone. If the economy improves, so does the construction business.

You have heard that the company working with the university on this project, Genetic Products, plans to build a $20 million research/production facility. You have a good reputation in the local countryside and you should be able to get at least 10% of the construction bids for the plant. If this happens, you will need to hire 50% more people to do the work.

You see the construction of the plant having a great impact on the economy of the area as it will provide many jobs and increase the need for family home and business constructions in the future.

You think the politicians should get busy and do what it takes to get the field test approved and encourage the construction of the research production facility.

References

Antifrost bacteria: so far, so good. (1987, June 20). *Science News, 131* (1), 396.

Biotechnology to change farm landscape. (1986, April 12). *Science News, 129* (1), 15, 233.

Bogley, Sharon. (1984, Nov. 12). Greening the gene: Biotechnology is sowing a crop of brave new plants, (economical aspects of genetic engineering). *Newsweek, 104* (2), 103.

Fenyveswi, Charles. (1986, July 14). Federal rules may trigger biotech boom. *U.S. News & World Report, 102* (1), 58.

Genes, not jeans (biotechnology may change agricultural economics). (1988, May). *Scientific American, 254* , 66.

Market projections. (1987, November). *BIO/Technology,* p1109.

Plant biotechnology to play key role in agriculture. (1986, May 26). *European Chemical News, 46* (1), 15.

Smithy, Emily T. (1987, Feb. 23). Bacteria + biotech = heartier harvests. *Business Week,* (1), 122.

Wessman, L. (1986, June 2). Davis' Calgene will take its plant genes public (company profile). *Business Journal* Serving Greater Sacramento, *3*, 8.

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Dr. Mogumbi

Plant Pathologist (Pro-Test)

You are a plant pathologist who believes the experiment proposed by Genetic Products poses no special risks to the environment. This field test appears to be less risky than experiments already completed that don't require **any** approval at all! There is no prohibition against putting mutagenized or genetically changed cells into the environment except for those involving recombinant DNA procedures.

Gene altering is done all the time. The brewing of beer, fermentation of wine, cheese, yogurt – while not genetically engineered -- the organisms used to make these products **are** genetically altered because of human intervention. The genetic alteration comes from continuously selecting the microbial strains that give the best product. The choice of microbial strains arises because microbes mutate with a predictable frequency even in the absence of known mutagens.

You feel the use of gene splicing techniques will actually allow more control over the diversity and specificity of genetic alteration than classical methods.

References

Government regulations criticized (genetic engineering research hampered by regulations). (1986, June). *USA Today , 114* ,15.

Mr./Ms. Schroeder

County Resident (Pro-Test)

You are a farmer and have lived in the county all of your life. You have read the results of the draft Environmental Impact Report (EIR) and believe the Ice-minus experiment poses neither health nor environmental hazards.

You don't really understand all the technical terms of the EIR, but you do realize that this is an experiment that could possibly change your life.

You own a farm near the test site where you grow potatoes, grain, strawberries and peaches. Five years ago, your farm was very productive, but the profits have declined in the past two years due to early spring frosts which have reduced your yields to 50% of their former levels. If this experimental Ice-minus bacteria does what the university spokesman says it will do, you could very well see productivity back to the level it was five years ago. This could mean the difference in whether you have to sell the farm or continue in the profession you dearly love.

It worries you that the tests are being done so close to your farm. You feel, though, that the university has been a good citizen and a good advisor to the farmers in the past and would not test something unless it was safe for everyone. You think the test should continue but it would definitely ease your mind if they moved the test site a little farther from your farm.

References

Antifrost bacteria: so far, so good. (1987, June 20). *Science News, 131*, 396.

Biotechnology to change farm landscape. (1986, April 12). *Science News, 129* (1), 233.

Rhein, R. Jr. (1985, January 14). Splicing together a regulatory body for biotechnology (need for federal guidelines in biotechnology industry). *Business Week*, 69.

Schnmeiderman, H.A. (1985, November). Altering the harvest: biotech's cornucopia (genetic engineering produces more food). *Science '85*, 52.

Sharples, F.E. (1987, March 13). Regulation of products from biotechnology (environmental applications and risk assessment). *Science, 235*, 1329.

Smithy, E. (1987, February 23). Bacteria + biotech = heartier harvests. *Business Week*, 122.

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Brief Summary of Con Characters

Dr. Carmazzi

Molecular Biologist & President, Applied DNA Research, Inc.

A conservative molecular biologist. You feel that the release of *P.syringae* poses some environmental risk.

Mr./Ms. LaPier

School Board Member

Member of the Board of Education and against the test. In the report it is stated, "The field test may have potential practical value." Wonders why it should be done at all if it only **may** have practical value.

Mr./Ms. Greenberg

Chairperson, County Board of Commissioners

A member of a group of fruit growers who oppose the experimental testing and release of genetically altered bacteria. You are worried about its adverse effect on the marketability of the local fruit products.

Mr./Ms. Oakes

Retired Farm Advisor

Citizen concerned about adverse publicity that might affect the markets for strawberries and other fruit products of the town.

Mr./Ms. Hunt

State Representative

A politician who generally favors new technology, but strongly feels that caution is needed in unknown experimental waters.

Mr./Ms. Esposito

Farmer & Spokesperson, Municipal Committee of Concerned Citizens

Fears that public fears might lower the price of local fruit, or even make it unsalable.

Mr./Ms. Shane

President, Religious Council

Opposes genetic engineering on religious grounds. Sees it as tinkering with what God has made.

Mr./Ms. Arnold

High School Biology Department Chairperson

A science teacher who represents other teachers against the test. Opposes the test on environmental grounds.

Dr. Newman

Atmospheric Microbiologist

A microbiologist who has done research on the Ice-minus bacteria. One of the few scientists apart from the developers at Genetic Products who opposes the test.

Dr. Carmazzi

Molecular Biologist &
President, Applied DNA Research, Inc. (Anti-test)

You are a conservative molecular biologist. Because of differences with others, you have formed your own firm, Applied DNA Research, Inc., so that research can be conducted in the careful way you feel is necessary.

You have examined the information distributed by the Ice-minus developers and concluded that the mutant *P.syringae* bacteria the researcher wishes to release are novel organisms with no identical counterparts in nature. Because of this, you feel that the release of *P.syringae* poses some environmental risk. You don't believe anyone knows whether the genetically-engineered bacteria will be more viable than the present wild strain, and no one knows what the impact will be.

Since you are a researcher in molecular biology, you are aware of the regulations against casual release of mutant genes to the atmosphere. You feel that there needs to be more research on recombinant organisms to assure their harmlessness before release into the atmosphere.

References

Steinhart, P. (1985, March) Mighty microbes. *Audubon, 87,* 6.

Mr./Ms. LaPier

School Board Member (Anti-test)

You are a member of the Board of Education and against the test. You have read the Environmental Reports and are unsure of the bacteria's guaranteed safe use. In the report it is stated, "The field test may have practical value." You wonder why it should be done at all if it only **may** have practical value. You feel a strong responsibility about protecting the town that has elected you to lead the school. Even though the issue does not directly affect the education of the town's children, before the town approves this project, you believe you should be guaranteed some sort of return before you agree to a field test so close to home. You also feel that 50% of what Genetic Products gives the town should go toward education since it is the children who will likely be hurt from any negative results of the testing. Since no one has been willing to earmark any money for education, you are not willing to support the project.

References

DNA plant products won't be marketed nationally by Kraft. (DNA Plant Technology Corp.). (1987, June 29). *Wall Street Journal*, p22(W), p28(E) col 4.

Why investors are losing their biotechnology bug. (1984, December 4). *Economist, 293*, 89.

Mr./Ms.Greenberg

Chairperson, County Board of Commissioners (Anti-test)

You have joined with the group of strawberry growers who oppose the experimental testing and release of the genetically altered bacteria. You are worried about its adverse effect on the marketability of the locally grown crops. You are also concerned about the potential adverse effect on the economy of the fruit producers if the genetically-altered bacteria turns out to be harmful to the environment.

References

Battling biofundamentalists (includes article on American attitudes toward biotechnology). (1987, June 27). *Economist, 303* (3), 87.

Chamberland, Dennis. (1986, Feb. 7). Genetic engineering: promise & threat. *Christianity Today, 31* (7), 22.

Culliton, Barbara J. (1985, Oct. 25). Episcopal Church backs genetic engineering. *Science, 230* (1), 423.

DNA plant products won't be marketed nationally by Kraft. (DNA Plant Technology Corp.). (1987, June 29). *Wall Street Journal*, p22(W), p28(E) col 4.

Durbin, William A. I., Jr. (1986, Feb. 7). Should Christians oppose genetic engineering? *Christianity Today, 31* (2), 54.

Gabor, A. (1985, November 25). Fresh from the labs, a farming revolution. *U.S. News & World Report, 99*, 74.,

Genes, not jeans (biotechnology may change agricultural economics. (1988, May). *Scientific American, 254*(1), 66.

Market projections. (1987, November). *BIO/Technology*, 1109.

Miller, J. A. (1984, March 24). The clergy ponder the new genetics. *Science News, 125* (3), 188.

Steinhart, P. (1985, March) Mighty microbes. *Audobon, 87*, 6.

Tangley, L. (1987, July-August). Engineered bacterial released. *BioScience, 37*, 461.

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Mr./Ms. Oakes

Retired farm advisor (Anti-test)

You are concerned about unanswered questions about the Genetic Products proposed test of the Ice-minus Bacteria. You feel safe that the release of these bacteria pose a serious environmental threat.

You are also aware of how volatile the wholesale market is. You know how easily a farming community can lose the confidence of market wholesalers who can refuse to buy the products from the area. You are concerned about any adverse publicity that might affect the markets for strawberries and other agricultural products.

References

Battling biofundamentalists (includes aricle on American attitudes toward biotechnology). (1987, June 27). *Economist, 303* (3), 87.

Bugs in the system (risks and safeguards of releasing alterted genes into the environment). (1986, June). *Scientific American, 62*, 2.

Chamberland, Dennis. (1986, Feb. 7). Genetic engineering: promise & threat. *Christianity Today, 31* (7), 22.

Critser, G. (1986, January). Big companies taking over agriculture. *Inc., 8*, 17.

Culliton, Barbara J. (1985, Oct. 25). Episcopal Church backs genetic engineering. *Science, 230* (1), 423.

DNA plant products won't be marketed nationally by Kraft. (DNA Plant Technology Corp.). (1987, June 29). *Wall Street Journal*, p22(W), p28(E) col 4.

Durbin, William A. I., Jr. (1987, Sept. 4). Should Christians oppose genetic engineering? *Christianity Today, 31* (2), 54.

Gabor, Andrea. (1985, November 25). Fresh from the labs, a farming revolution. *U.S. News & World Report , 99* (2), 74.

Miller, J. A. The clergy ponder the new genetics. *Science News, 125*(3), 188.

Schneiderman, H.A. (1985, November 6). Altering the harvest: Biotech cornucopia (genetic engineering produces more food). *Science* '85, *52*.

Steinhart, P. (1985, March) Mighty microbes. *Audubon, 87*, 6.

Tangley, Laura. (1986, Nov.). Agricultural biotechnology: Who's holding the reins? Critics claim that control is concentrated in too few hands (part 2). *BioScience, 36*, (4) 652.

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Mr./Ms. Hunt

State House of Representatives
(Anti-test)

You have been contacted by several of your constituents regarding the altered bacteria experiment. You generally favor new technology, but you strongly feel that considerable caution is needed in these unknown experimental waters. You hear from your constituents that many of them are worried. Generally, since it is such a new and controversial research area, they are urging you to do what you can to avoid the testing until they can be sure it is safe.

Regardless of the university's convictions that the experiment is safe, it is plainly apparent that those convictions are not shared by the general public. You feel that in view of the current economic and social environment, conducting the experiment is irresponsible.

References

Ban experiments in genetic engineering? (interview with Jeremy Rifkin, Bernard Davis). (1984, Oct. 8). *U.S. News & World Report, 97*, (1) 44.

Biotechnology gets good marks in survey. *Chemical & Engineering News, 63* (1), 16.

Blueston, M. (1987, June 15). How squeamish are we about genetic engineering? (Harris poll on public opinion of genetic engineering). *Business Week*, 65.

Bugs in the system (risks and safeguards of releasing altered genes into the environment). (1986, June). *Scientific American, 62*, 2.

Critser, G. (1986, January). Big companies taking over agriculture. *Inc., 8*, 17.

Genes, not jeans (biotechnology may change agricultural economcs). (1988, May). *Scientific American, 254* (1), 66.

Goldbaum, Ellen. (1987, June 10). Poll results may bode well for biotechnology. *Chemical Week, 140* (2), 15.

Hanson, David. (1987, June 15). Survey finds public supports biotechnology. (1985, Aug. 16). *Chemical & Engineering News, 65* (1), 22.

Miller, Jon D. (1987, June). Scientifically illiterate (research on adults' understanding of scientific terms and concepts). *American Demographics, 9* (6), 26.

Rogers, Michael. (1986, May 26). Tinkering with nature; genetic engineering may revolutionize agriculture -- but is it safe? *Newsweek, 107*, (3), 54.

Sharples, F.E. (1987, March 13). Regulation of products from biotechnology (environmental applications and risk assessment). *Science, 235*, 1329.

Sun, M. (1986, February 14). Local opposition halts biotechnology test. *Science, 231*, 667.

Tangley, L. (1985, May). New biology enters a new era; a decade after a historic vote to restrict research, recombinant DNA technology promises fresh rewards – as well as problems. *BioScience, 35* , 270.

Tangley, L. (1985, September). Releasing engineered organisms in the environment; assessing the risks of genetically engineered organisms will require closer collaboration between molecular biologists and ecologists. *BioScience, 35*, 470.

Mr./Ms. Esposito

Farmer & Spokesperson, Municipal Committee of Concerned Citizens (Anti-test)

You are a strawberry farmer who is against the experiment. You are afraid that the consumers might refuse to buy local strawberries. If environmental problems creep into the situation, in addition to your other economic woes, you will most likely be forced to sell your farm.

You have joined forces with other concerned farmers and citizens. You want to avoid this testing unless absolute guarantees can be given that your crops will not suffer as a result of the proposed testing.

References

Battling biofundamentalists (includes article on American attitudes toward biotechnology). (1987, June 27). *Economist, 303*, 87.

Chamberland, D. (1986, February 7). Genetic engineering: Promise & threat. *Christianity Today, 31*, 22.

Culliton, B.J. (1985, October 25). Episcopal Church backs genetic engineering. *Science, 230*, 423.

Durbin, W.A.I. Jr. (1987, September 4). Should Christians oppose genetic engineering? *Christianity Today, 31* , 54.

Frost-free bacteria lawsuit (genetic engineering). (1985, March 2). *Science News, 127*, 135.

Gabor, A. (1985, November 25). Fresh from the labs, a farming revolution. *U.S. News & World Report, 99*, 74.

Howard, L. (1986, February 10). Halting designer bacteria (testing of Frost ban on Monterey crops). *Newsweek, 10*, 8.

Miller, J.A. (1984, March 24). The clergy ponder the new genetics. *Science News, 125*, 188.

Rhein, R. (1986, April 7). The EPA locks gene-splicers in the lab (biotechnology company violations). *Business Week, 293* , 42.

Sun, M. (1986, February 14). Local opposition halts biotechnology test. *Science*, 231, 667.

Tangley, L. (1985, September). Releasing engineered organisms in the environment; assessing the risks of genetically engineered organisms will require closer collaboration between molecular biologists and ecologists. *BioScience, 35*, 470.

Mr./Ms. Shane

President, Religious Council
(Anti-test)

You are the president of the lay council for all churches in the area. You represent the position and the sentiments of all the group members. You oppose genetic engineering on religious grounds. You see it as tinkering with what God has made. You want the town council to take a moral stand and not allow any biotechnology plant to be built in your town. You believe the people of the town are conservative, and this is what they want.

References

Battling biofundamentalists (includes article on American attitudes toward biotechnology). (1987, June 27). *Economist, 303*, 87.

Chamberland, D. (1986, February 7). Genetic engineering: Promise & threat. *Christianity Today, 31,* 22.

Culliton, B.J. (1985, October 25). Episcopal Church backs genetic engineering. *Science, 230,* 423.

Durbin, W.A.I. Jr. (1987, September 4). Should Christians oppose genetic engineering? *Christianity Today, 31* , 54.

Miller, J.A. (1984, March 24). The clergy ponder the new genetics. *Science News, 125,* 188.

Mr./Ms. Arnold

High School Biology Department Chairperson (Anti-test)

You are a science teacher who represents other teachers in the area who are against this plant. You have some molecular biology training. You oppose the test on environmental grounds.

You are also aware of other efforts to control the environment that have had disastrous consequences for the environment. You are prepared to explain to the Town Council members about the effects of efforts to control the environment such as using DDT to control insects on crops. When this pesticide was introduced, no one thought it would harm the environment. Yet, the consequences for wildlife have been monumental and long-term. You and the group you represent do not want another disaster to arise because scientists do not know the long-term effect of what they are doing. What about weeds that this bacteria protects from the frost? What will happen when **nothing** dies in the frost?

References

Organic Gardening. (1984, July). 65.

Sharples, F.E. (1987, March 13). Regulation of products from biotechnology (environmental applications and risk assessment). *Science, 235,* 1329.

Strawberry fields forever (genetically altered organisms will be allowed into the environment). (1985, November 11). *Time, 126,* 74.

The Ice-Minus Case

STUDENT PAGE 33

Dr. Newman

Atmospheric Microbiologist
(Anti-test)

You are currently a microbiologist at a leading university. You have done extensive research on the Ice-minus bacterium controversy in order to write your dissertation on the subject. You are one of the few scientists apart from the developers at Genetic Products who has investigated this research.

Some time ago, you were asked to investigate the potential atmospheric consequences of use of the Ice-minus bacterium. Research has been conducted in the laboratory to investigate Ice-minus, but not in the field or in the atmosphere. There is a concern on the part of some that introduction of this bacterium into the environment may permanently alter the local atmospheric and metrological conditions. There is also the concern that prevailing winds may carry the bacterium throughout the state and the region and have adverse effects over a wide-spread area.

You designed and tested a study to test the atmospheric consequences of using the Ice- bacterium. Despite extensive experimentation, your results are inconclusive. You are unable to state conclusively whether the bacteria likely will or will not have harmful effects.

References

Brill, Winston J. (1985, Jan. 25). Safety concerns and genetic engineering in agriculture. *Science*, f227 (4), 381.

Kozloff, L.M., Schofield, M.A. & Lute, M. (1983). Ice nucleating activity of *Pseudomonas syringae Erwinia herbicola*. *Journal of Bacteriology, 153*, 222-231.

Levin, Z., Sandlerman, N., Moshe, A., Bertold T. & Yankofsky, S.A. (1980). Citrus derived bacteria as freezing nuclei at -2.5 C. Proceedings of the International Conference on Cloud Physics (pp.45-47). Clemont-Ferrand, France.

Lindemann, J., Constantinidou, H.A., Barchet, W.R. & Upper, C.D. (1982). Plant as sources of airborne bacteria including ice nucleation-active bacteria. *Applied Environmental Microbiology, 44*, 1059-1063.

Maki, L.R., Galyan, E.L., Chang-Chien, M.M., and Caldwell, D.R. (1974). Ice nucleation induced by *Pseudomonas syringae*. *Applied Microbiology, 28*, 456-459.

Maki, L.R. & Willoughby, K.J. (1978). Bacteria as biogenic sources of freezing nuclei. *Journal of Applied Meteorology, 17*, 1049-1053.

Schnell, R.C. & Vali, G. (1972). Atmospheric ice nuclei from decomposing vegetation. *Nature, 236*, 163-165.

Schnell, R.C. & Vali, G. (1973). Worldwide source of leaf-derived freezing nuclei. *Nature, 246*, 212-213.

Schnell, R.C. & Vali, G. (1976). Biogenic ice nuclei: Part I. Terrestrial and marine sources. *Journal of Atmospheric Science, 33*, 1554-1564.

Schnell, R.C. (1979). A new technique for measuring atmospheric ice nuclei active at temperatures from -20C to approaching 0C, with results. Preprints for Seventh Conference of Inadv. and Planned Weather Modif. (pp. 110-111). American Meterological Society, Boston, MA.

Vali, G. (1971). Freezing nucleus content of hail and rain in Alberta. *Journal of Applied Meteorology, 10*, 73-78.

Vali, G., Christensen, M., Fresh, R.W., Galyan, E.L., Maki, L.R. & Schnell, R.C. (1976). Biogenic ice nuclei. Part II: Bacterial sources. *Journal of Atmospheric Science, 33*, 1565-1570.

Vali, G. (1978). Freezing nucleus content of hail and rain in N.E. Colorado (monograph). *American Meterological Society, 38*, 93-105.

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Glossary

Antibiotic - a substance that can kill or inhibit the growth of various kinds of bacteria. Natural antibiotics are secreted by fungi or bacteria.

Antigen - a substance (usually a protein) that causes the immune system to produce antibodies.

Bacteriophage - a virus that infects bacteria by injecting its DNA or RNA into a bacterium. In experiments, bacteriophage DNA or RNA is recombined with a desired gene, and the bacteriophage places the recombinant DNA into bacteria.

Biotechnology - a collection of techniques that use living organisms to make products or solve problems. The term most commonly refers to the biological techniques resulting from recent discoveries about the chemistry of cells. The techniques include genetic engineering, diagnostics, and cell/tissue culture.

Cell culture - a technique for growing or cloning cells *in vitro*.

Cell fusion - the combining of two or more cells to form a single cell. Cell fusion is used to make monoclonal antibodies.

Chromosome - the filamentous structure found in the cytoplams of prokaryotic cells and the nucleus of eukaroytic cells. Chromosomes are composed of the molecule DNA. The units of chromosomes are called genes.

Clone - a cell or collection of cells containing identical genetic material. Clones are produced from a single parent cell by asexual reproduction. **To clone**: To produce a number of genetically identical individuals. In genetic engineering, this process is used to make a great number of identical DNA molecules, viruses, cells or tissues.

DNA - the large, double-stranded, complex molecule that is the basic genetic material found in all viruses or cells. DNA provides the blueprint for making proteins, and it carries hereditary information from one generation to the next. The DNA molecule is universal for all forms of life so that DNA taken from any organism can be inserted into another organism. DNA stands for deoxyribonucleic acid.

DNA ligase is an enzyme that rejoins cut pieces of DNA.

E. coli - a bacterium commonly found in the human intestine and used extensively in recombinant DNA research.

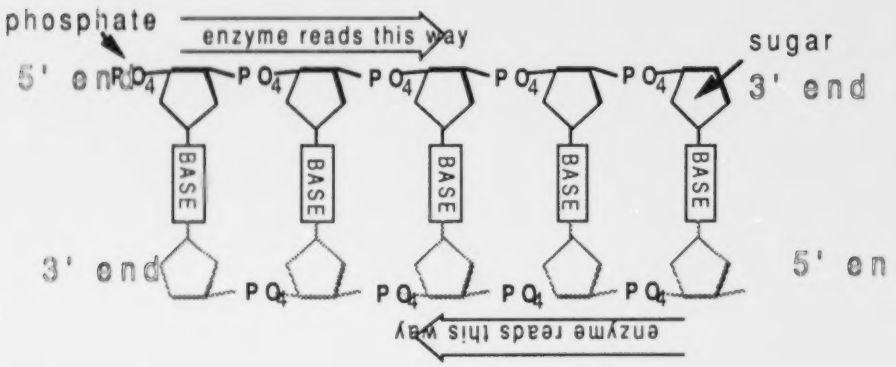
Endonucleases - restriction enzymes that cut DNA at specific points *between its ends*, (exonucleases digest DNA from its ends). Endonucleases consistently cut DNA into fragments of predictable lengths, providing a valuable tool for studying and recombining DNA. Endonucleases naturally occur in bacteria to protect them from the effects of foreign DNA.

Enzyme - a protein that accelerates the rate of chemical reactions. Enzymes in saliva begin the chemical reactions that digest food. Enzymes are catalysts. An enzyme promotes a reaction over and over again without being changed by that reaction.

Eukaryote - a cell with a membrane around its genetic material; this structure is called the nucleus. Eukaryotes also have membranes around other structural and functional units in the cell called organelles.

Glossary

Five prime ends (5') are the ends of DNA fragments where restriction enzymes begin their "reading" process. The 5' end is the the phosphate end of the DNA fragment. The number 5 refers to the fifth carbon atom in the sugar molecule. When the bond between the phosphate and the sugar breaks in a DNA molecule, the phosphate remains attached to the 5' end (See below.)



Gene - the smallest unit of inheritance that passes from one generation to the next. Genes are DNA sequences that code for a single protein. The DNA sequences in a single gene *do not* have to be a single continuous stretch of DNA.

Genetic code - the code that stores the information for all genetically determined characteristics. The code is a sequence of nucleotides taken in groups of three. The code is expressed through protein synthesis: The sequence of amino acids in a protein by the sequence of DNA or RNA in the cell or virus.

Genetic engineering - the manipulation of the genetic material in a cell to produce a desired trait; sometimes called recombinant DNA.

Genetic diversity - the variety of gene forms (alleles) that are available to produce traits in a breeding population. Genetic diversity is a measure of a species' ability to respond to selection pressures and its ability to adapt.

Genome - the amount of genetic material needed to replicate an entire organism or virus.

Genotype - the invisible genetic make-up of an organism or cell that shapes visible traits (see phenotype). Genotype is determined by the DNA nucleotide sequence in the genome.

Hybridoma - a type of hybrid cell produced by fusing a normal cell with a tumor cell. When lymphocytes (antibody-producing cells) are fused to the tumor cells, the resulting hybridomas produce antibodies and maintain rapid sustained growth, producing large amounts of antibody. Hybridomas are the source of monoclonal antibodies (see monoclonal antibody).

In-vitro - any biological event or reaction that occurs outside of a living organism. Usually, parts of an organism are placed in artificial conditions such as a nutrient medium on a glass plate.

In-vivo - any biological event investigated within the living organism.

Interferon - a protein that helps the immune system fight viral infections. Interferon is naturally produced by animal cells in response to viral infection.

Micropropagation - a process for propagating plants from individual cells. The process involves removing fast-growing meristem tissue, isolating individual cells and growing those cells as clones of the parent.

Glossary

Monoclonal antibody - an antibody produced by culturing hybridoma cells (see hybridoma). The word monoclonal refers to the fact that the antibodies are produced from a single line (see clone) of hybridoma cells. The cells produce antibodies for a specific antigen (see antigen) because the lymphocyte component of the hybridoma has been stimulated to produce antibodies for a specific antigen.

Mutation - any change in DNA or viral RNA. Mutations can occur at the nucleotide, gene or chromosome level. Sometimes mututions are expressed as a new or abnormal characteristic.

Nucleotide - the basic building blocks of DNA and RNA. Nucleotides consist of a nitrogenous base, a pentose sugar, and phosphoric acid.

pAMP - a plasmid containing a gene that, when expressed, confers ampicillin resistance on bacteria.

Patent - legal restrictions securing the exclusive right to make use or sell a product for a specified term. Patents protect individuals' or companies' research investments. To be eligible for patenting, living organisms must have characteristics produced by man that are verifiably distinct from other organisms.

Phenotype - the visible or measurable characteristics of an organism brought about by the expression of genes.

pKAN - a plasmid containing a gene that, when expressed, confers kanamycin resistance on bacteria.

Plasmid - a small circular piece of bacterial DNA separate from the bacterial chromosome, usually containing from 1-3% of the cell genome. Plasmids can be absorbed into living bacteria, thus carrying genes into a living system.

Prokaryote - an organism without a membrane around its genetic material or any other intracellular structure; bacteria and blue-green algae are the two major groups of prokaryotes.

Protein - a relatively large molecule composed of chains of amino acids. Proteins serve many functions in living systems. Examples of proteins are enzymes, hormones and antibodies. Proteins can also function as nutrient and waste transporters, nutrient storers or as a source of energy for cells and tissues.

Protoplast - a plant cell whose cell walls have been removed by enzymes. A protoplast is the total contents of a plant cell excluding everything outside of the plasma membrane.

Protoplast fusion - a process that fuses two protoplasts, including their genetic material, into a single hybrid cell. The hybrid cells are stimulated to regrow a cell wall and then generate a new plant.

Recombinant DNA (rDNA) - segments of DNA from two different organisms spliced together to form a new molecule. The rejoined piece of DNA is placed into a host organism so that the host organism gains traits it does not normally have. rDNA is sometimes referred to as genetic engineering.

Restriction digest - the use of restriction enzymes to cut DNA into fragments.

Restriction enzymes are enzymes that cut DNA at specific base sequence sites. Examples: EcoRI, BamHI, HindIII. Restriction enzymes are *endonucleases*.

RNA - a complementary, single-stranded nucleic acid to DNA that is mostly used by cells to make proteins. There are three major types RNA: mRNA decodes the DNA message and takes it to the site of protein synthesis in the cell or virus; tRNA attaches to amino acids in the cell and attaches to mRNA; rRNA is a major component of ribosomes, but its function is not totally understood yet. RNA is also the major nucleic acid in some types of viruses that do not have DNA.

Sticky ends - uneven ends of DNA after it is cut by a restriction enzyme. These ends are chemically sticky because they attach to the ends of other DNA fragments.

Glossary

Three prime ends (3') are the sugar ends opposite the 5' ends of DNA molecules (see Five prime ends above)

Tissue culture - a procedure for growing or cloning enough cells through *in-vitro* techniques to make a tissue.

Transformation - a genetic change induced in a cell resulting from the uptake and incorporation of foreign DNA.

General Resources

Journal and Newspaper Articles

Agriculture

Basgall, M. Pine clones offer shortcut for tree breeders. (1988, March 3). *Raleigh News and Observer*, p. 12.

Biotech issues for vets discussed at forum. (1988, Nov/Dec). *AgBiotech News*, pp. 5,16.

Biotechnology could begin green revolution in the fields. (1988, June 14). *Durham Sun*, n.p.

Cowen, R.C. New wave crops heading for the farm. (1988, June 1). *The Christian Science Monitor*, pp. 16-17.

Cowen, R.C. Value added animals: What is on the horizon? (1988, June 2). *Christian Science Monitor*, p. 16.

Fackleman, A. Cancer-fighting tobacco plants? (1989, April). *Science News*, *135*(15), p. 238.

Freiberg, B. Biotechnology: Slow, incremental progress. (1988, April). *Seedsmen's Digest*, pp. 16-21.

Gene may protect beans from weevils. (1988, April 19). *New York Times*, p. 28.

Gladwell, M. USDA's chicken feat: A resistant new breed. (1988, August 11). *Washington Post*, p. E3.

Gould, F. Evolutionary biology and genetically engineered crops. (1988, January). *BioScience*, *38*(1), n.p.

Laboratory animal market sparks new interest in transgenic animals. (1989, February 8). *Biotechnology News*, *9*(4), pp. 4-6.

Mazzola, V. High tech lends helping hand to cattle producers. (1988, June/July). *Agricultural Research*, p. 7-10.

Meyer, B. Seed scientists predict changes with biotech. (1988, June). *Seed Industry*, pp. 26-27.

Protecting poultry from viral infections: New research initiated. (1989, March). *New Biotechnology Business*, *1*(20), pp. 8-9.

Rensberger, B. Plant gene fights herbicide. (1988, October 31). *The Washington Post*, p. A3.

Spalding, B.J. The big plant biotechnology shootout. (1988, August). *Chemical Week*,*143* (9). p. 16.

Strange creatures - entering an age of engineered animals. (1987, August). *Science Impact Letter*, *1*(3), p. 1-2.

The green (gene) revolution. (1988, May). *Science Impact Letter*,*1*(12), pp. 1-2.

Transgenic poultry - lab produces chimeric chicks. (1988, April). *New Biotechnology*, *2*(7), p. 15.

Weiss, R. The revolution will not be fertilized. (1989, January). *Science News*, *135*(4), p. 50.

Wheeler, D. Agricultural scientists step up efforts to find biological weapons against crop pests grown resistant to chemical insecticides. (1988, August). *Chronicle of Higher Education*, *34*(47), pp. A4, A5, A10.

Aquaculture

Meyer, B. Aquaculture gaining more respect. (1988, September/October). *AgBiotechnology News*, *5*(5), pp.3, 20-21, 23.

Miller, J.P. Search for new drugs focuses on organisms under the sea. (1987, July 24). *Wall Street Journal*, p.23.

Morrison, D. Regulatory uncertainty threatens development of transgenic fish. (1988, July). *Water Farming Journal*, p. 6,7.

Schneider, K. Insertion of trout gene yields faster-growing type of carp. (1988, June 2). *Raleigh News & Observer*, p. 1A.

Superfish: Is there one in your future? (1988, June). *Water Farming Journal*, *3*(5), p. 14.

Will catfish pass human gene to its offspring? (1988, September). *Water Farming Journal*, *3*(8), pp. 1, 6.

Biotechnology Education

Dixon, L. Teaching recombinant DNA technology in high school biology courses. (1988, September). *The American Biology Teacher*, *50*(6), pp. 368-373.

Ehrman, P. A user-friendly method for teaching restriction enzyme mapping. (1990, October). *The American Biology Teacher*, *52*(7), pp. 429-435.

Gardner, A.M. Biotechnology in 3 days. (1988, October). *The American Biology Teacher*, *50*(7), pp. 446-448.

Guilfoile, P. A simple DNA isolation technique using halophilic bacteria. (1989, March). *The American Biology Teacher*, *51*(3), pp.170-171.

Luken, J.O. Does biotechnology have a place in introductory biology? (1987, September). *The American Biology Teacher*, *49*(6), pp. 351-354.

Mertens, T.R. & Pursifull, J. The gene scene: A human genetics game. (1986, February). *The American Biology Teacher*, *48*(2), pp. 104-108.

Myers, R. A method for isolating DNA from E. coli. (1985, September). *The American Biology Teacher*, *47*(6), pp.362-364.

Myers, R. Recombinant DNA technology in the high school biology laboratory. (1988, January). *The American Biology Teacher*, *50*(1), pp. 43-45.

Oppenheimer, S.B., Maxwell, J.B. & Allen, L.G. Advances in biological science. (1988, January). *The American Biology Teacher*, *50*(1), pp. 18-22.

Peebles, P. & Leonard, W. H. A hands-on approach to teaching about DNA structure and function. (1987, October). *The American Biology Teacher*, *49*(7), pp. 436-458.

Seidel-Rogol, B.L. An exercise in gene mapping: Using hybridization analysis to detect a restriction site polymorphism in the rDNA gene of Neurospora crassa. (1990, February). *The American Biology Teacher*, *52*(2), pp. 103-106.

Sigismondi, L.A. A paper model of DNA structure and replication. (1989, October). *The American Biology Teacher*, *51*(7), pp. 422-423.

Thomson, R.G. Recombinant DNA made easy I: "Jumping genes". (1988, February). *The American Biology Teacher*, *50*(2), pp. 101-106.

Thomson, R.G. Recombinant DNA made easy II: Gene, gene, who's got the gene? (1989, April). *The American Biology Teacher, 51*(4), pp. 226-232.

Vigue, C.L. Murphy's Law and the human beta-globin gene. (1987, February). *The American Biology Teacher, 49*(2), pp. 76-81.

DNA Fingerprinting

DNA identification systems and civil liberties: An interview with Philip Bereano. (1989, May/June). *geneWATCH, 5*(6), pp. 1-3, 8-9.

Forensics 1989: The case of the unraveling DNA. (1990, January). *Discover*, pp. 46-47.

Fox, J. Improved DNA analysis may be mixed blessing. (1988, May). *Bio/Technology, 6*(5), pp. 459-60.

Kelly, K.F., Rankin, J.J. & Wink, R.C. Method and applications of DNA fingerprinting: A guide for the non-scientist. (1987). *The Criminal Law Review*, pp. 105-110.

Lewin, R. DNA fingerprints in health and disease. (1986, July-September). *Science*, 233., pp. 521-22.

Lewis, R. DNA fingerprints: Witness for the prosecution. (1988, June). *Discover, 9*(6), pp. 44-52.

Sensabaugh, G.F. Forensic biology — Is recombinant DNA technology in its future. (1986). *J. Forensic Science, 31*, pp. 393-96.

Thompson, L. Genetic clues to solving a crime. (1989, December 26). *The Washington Post (Health)*, p. 7.

Environmental Release

Cowen, R.C. Regulating biotech: How much is enough? (1988, June 3). *Christian Science Monitor*, pp. 16-17.

Davis, B.D. Exposing fatal flaws in some arguments against recombinant release. (1988, February). *Genetic Engineering News, 8*(2), pp. 20-21.

Maugh, T.H. Biotech bugs leave the lab - at last. (1988, January). *Discover*. pp. 84-85.

Offutt, S. & Kuchler, F. Biotechnology - is safety all that matters? (1987, fourth quarter). *Choices*, pp. 12-15.

Spalding, B.J. Making bugs that self-destruct. (1988, May). *Chemical Week, 142*(19), p. 39.

Study discounts genetic test risk. (1988, May 5). *New York Times*, p. 11.

Weiss, R. Engineered microbes stay close to home. (1988, February). *Science News*, p. 117.

Ethics

Kormondy, E.J. Ethics and values in the biology classroom. (1990, October).
The American Biology Teacher,52(7), pp.403-407.

Nicoll, C.S. & R.S. Animal research vs. animal rights. (1989, March). *FASEB Journal, 3*(5), pp. 1668-1671.

Singer, M. Biology frontiers pose ethics questions. (1988, July). *NRC News Report*, pp. 19-21.

Wallis, C. Should animals be patented? (1987, May 4). *Time*, n.p.

Weiss, R. Forbidding fruits of fetal-cell research: Ethical issues raised by promising therapy. (1988, November). *Science News, 134*(19), pp. 296-298.

Williams, E. Ethicist warns of taking genetic tinkering too far. (1988, July 1). *Greensboro News & Record*, pp. C1, C2.

Medicine

Beale, A.B. Biotechnology: Impact on immunisation. (1983, July). *Veterinary Record*, p. 77.

Booth, W. Scientists advance gene-implant technique in mice. (1990, April 19). *The Washington Post*, p. A22.

Buderi, R. Controversy surrounds gene therapy effort. (1989, January). *The Scientist, 3*(2), pp. 1-2.

Bylinsky, G. Coming: Star Wars medicine. (1987, April). *Fortune*, n.p.

Chase, M. Human gene transplants closer to reality as researchers pursue bid for experiment. (1988, July 13). *Wall Street Journal*, n.p.

Chu, M.L. & Kamely, D. Environmental cancer and biotechnology. (1988, June). *Bio/Technology, 6*(6), pp 675-678.

Freundlich, N. Patient, heal thyself - with lab grown cells. (1989, March 20). *Business Week*, pp. 148, 151.

Gadsby, P. Where AIDS takes aim. (1989, January). *Discover, 10*(1),
pp. 52-53.

Gene researcher cites gains toward curing inborn diseases. (1988, August 22). *Raleigh Times*, n.p.

Gene-spliced hormone hints of safer infertility treatment. (1988, June 10). *Greensboro News & Record*, p. A15.

Gladwell, M. New drug for "bubble boy" disease. (1990, March 24). *The Washington Post*, p. A3.

Gorner, P. Scientists seek to release rabies vaccine in wild. (1988, June 2). *Raleigh News & Observer*, n.p.

Graff, G. Custom tailored for immunity without side effects. (1988, May 15). *New York Times*, p. 13.

Graff, G. Interesting numbers for the manufacturers. (1988, May 15). *New York Times*, p. 13.

Hamilton, J. A cancer fighter starts showing its mettle. (1989, January). *Business Week, 3087*, pp. 96-97.

Jaret, P. Our immune system: The wars within. (1986, June). *National Geographic,*
169(6), pp. 702-735.

Kolata, G. New technique may create decoy to halt spread of AIDS. (1987, December 18). *The New York Times*, pp. 1, 14.

Kolata, G. Scientists try blocking cancer cells. (1988, April 27). *Raleigh News & Observer*, n.p.

Lab seeks to test gene-spliced drug. (1988, May 4). *New York Times*, p 17.

Lewis, R. Ribi ImmunoChem research: Helping the body fight cancer. (1987, January). *High Technology*, pp. 12,14.

Living cells as therapeutics - the next generation of pharmaceuticals? (1989, February). *BioTechnology News, 9*(3), pp. 6-8.

Marsh, P. Artificial skin offers an alternative to tests on animals. (1989, January 11). *Financial Times*, p. 10.

McInerney, J.D. Genetics and the quality of life. (1989, May). *The American Biology Teacher, 51*(5), pp. 264-268.

Merz, B. Biotechnology's bequest: New drugs, diagnostics. (1987, September). *Medical News & Perspectives, 258*(11), p. 1435.

Montgomery, G. The ultimate medicine. (1990, March). *Discover*, pp. 61-68.

New type of AIDS drug holds promise of letting immune system kill virus. (1989, February). *The Washington Post*, pp. A1, A4.

Okie, S. Study finds drug effective against cystic fibrosis. (1990, April 26). *The Washington Post*, p. A4.

Oppenheimer, S. Advances in cancer biology. (1987, January). *The American Biology Teacher, 49*(1), pp. 11-15.

Patlak, M. AIDS watch - one small step toward a vaccine. (1988, August). *Discover, 9*(8), p. 22.

Potash, M.J. Designing a vaccine taxes research ingenuity. (1988, February). *NRC News Report*, pp. 5-7.

Reese, K. M. Caterpillars may be used widely to make proteins? (1988, August). *Chemical & Engineering News, 66*(3), p. 156.

Researchers study AIDS in horses. (1988, January 17). *High Point Enterprise*, p. B7.

Revkin, A.C. The search for an animal model. (1988, November). *Discover, 9*(11), pp. 6-7.

Roberts, L. New targets for human gene therapy. (1988, August). *Science, 241*(4868), p. 906.

Schmeck, H.M. Gene technique is used to correct a liver defect. (1988, June 16). *New York Times*, p. 10.

Schmeck, H.M. Virus that beat smallpox is recalled to battle. (1988, August 2). *New York Times*, p. 17+.

Siegel, L. Arthritis gene discovered in mice gives victims hope. (1988, May 3). *High Point Enterprise*, n.p.

Slow but steady progress for human gene therapy. (1988, May). *Genetic Technology News*, p. 3.

Specter, M. Mice bred with disease like AIDS. (1988, June 15). *Washington Post*, p. A14.

Squires, S. Genetic testing for cystic fibrosis. (1989, October 31). *The Washington Post (Health)*, pp. 8-9.

The telltale gene. ((1990, July). *Consumer Reports*, pp. 483-488.

Thompson, L. Human test of gene altered cells proposed. (1988, June 15). *Washington Post.*, p A1.

Weiss, R. Gene therapy gets a boost with "natural" regulators. (1988, January). *Science News*, p.23.

Weiss, R. Improving the AIDS test - genetic engineers offer a new approach to AIDS-antibody testing. (1988, April). *Science News,133*(14), pp. 218-221.

Patents

Agres, T. Archaic patent file system strangles U.S. inventiveness. (1989, January). *Research and Development*, pp. 23-24.

Booth, W. Animals of invention. (1988, May). *Science, 240*(4853), p. 718.

Corcoran, E. Patent medicine. (1988, September). *Scientific American, 259*(3), pp.128, 130.

Schneider, K. Harvard gets patent on animal - a first. (1988, April 13). *Raleigh News and Observer*, pp. 1A, 10A.

Watters, J. National research council plays a major role in Canadian biotechnology. (1989, February). *Genetic Engineering News, 9*(2), pp. 12, 38.

Weiss, R. Animal patent report lacks support. (1988, April). *Science News*, p. 231.

Public Understanding

Basgall, M. Oh, brave new animal farm - will genetic engineers improve upon nature - or upset it? (1988, May 15). *Raleigh News and Observer*, pp. 1D, 8D.

Blank, R.H., Caldwell, L.K., Wiegele, T.C. & Zalinskas, R.A. Toward better education in biopolitics. (1988, Spring). *Issues in Science and Technology, 4*(3), pp. 51-53.

Dalton, D. Genetic gerrymandering. (1988, January/February). *The Humanist.*, n.p.

Editorially speaking: Don't fear biotechnology. (1988, July/August). *NC Farm Bureau News, 59*(7), p. 3.

Haskins, P. Biotechnology: A bitter harvest for traditional farmers? (1988, May 8). *Winston-Salem Journal*, n.p.

Regulation urged of genetically engineered plants. (1988, September 2). *Durham Morning Herald*, p. 4C.

Schneiderman, H. Genetic engineering. (1988, February). *Chemical and Engineering News*, p.3.

Weaver, R.F. Beyond Supermouse: Changing life's genetic blueprint. (1984, December). *National Geographic, 166*(6), pp. 818-847.

Why biotechnology may protect the environment. (1987, October). *Science Impact*, pp. 6-7.

Waste Disposal/Treatment

Bluestone, M., Schwartz, J. & Trewhitt, J. The quest for hungry genes. (1988, October 29). *Chemical Week*, pp. 38-39.

Bonitz, S. Hazardous waste fears challenge companies to find alternate solutions. (1988, October). *Genetic Engineering News, 8*(9), pp. 8-9.

Rotman, D. Degradable plastics are here to stay. (1988, October 26). *Chemical Week, 143*(17), pp. 38, 40, 42-43.

Silverman, E.R. Science steps up war on hazardous waste. (1989, January). *The Scientist, 3*(2), pp. 1, 4-6.

Miscellaneous

Biotech firm links microbe to gold fever. (1989, February). *Biotechnology News*, 9(2), pp. 2-3.

Booth, W. Ancient magnolia leaf yields strands of DNA. (1990, April 12). *The Washington Post*, p. A3.

Campanario, M. The energy equation. (1988, October). *Bio/Technology, 6*(10), pp.1139-1140.

Davis, J.D., Korschgen, A.J. & Saigo, B.W. Employment prospects in biotechnology. (1989, September). *The American Biology Teacher, 51*(6), pp. 346-348.

Gladwell, M. FDA approves bioengineered cheese enzyme. (1990, March 24). *The Washington Post*, pp. A1, A14.

White, R. & Lalouel, J. Chromosone mapping with DNA markers. (1988, February). *Scientific American, 258*(2), pp. 40-48.

Books

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J.D. (1983). *Molecular biology of the cell.* New York: Garlund Publishing.

Arthur Young High Technology Group. (1987). *Biotech 88: Into the marketplace.* California: Arthur Young.

Biological Sciences Curriculum Study. (1988). *Advances in genetic technology.* Lexington, MA: D.C. Heath and Company.

Biological Sciences Curriculum Study. (1980). *Biological science: A molecular approach* (4th ed.). Lexington, MA: D.C. Heath and Company.

Cherfas, J. (1983). *Man made life.* New York: Pantheon House.

Chevallier-LeGuyader, M. (1989). *Biology's building blocks.* Hauppauge, NY: Barron's Educational Series.

DeForest, P., Frankel, M.S., Poindexter, J.S. & Weil, V. (1988). *Biotechnology: Professional issues and social concerns.* Washington, D.C.: AAAS Publications.

Drlica, K. (1984). *Understanding DNA and gene cloning: A guide for the curious.* New York: John Wiley and Sons.

Fletcher, J. (1978). *The ethics of genetic control: Ending reproductive roulette.* New York: Anchor Press/Doubleday.

Friefelder, D. (1987). *Molecular biology* (2nd ed.). Portola Valley, CA: Jones and Bartlett Publishers.

Genetic engineering/recombinant DNA: A bibliography. (1982). Washington, D.C.: National Center for Education in Maternal and Child Health.

Hyde, M.O. & Hyde, L.E. (1984). *Cloning and the new genetics.* New Jersey: Enslow Publishers.

Iozzi, L. (1982). *Technology and society: A futuristic perspective* (a book of technology decision-making activities for high school students - contains a unit on biotechnology decision-making) Order from Sopris West, 1120 Delaware Ave., Longmont, CO, 80501.

Jackson, D.A. & Stich, S.P. (Eds.). (1979). *The recombinant DNA debate.* Englewood, NJ: Prentice Hall.

Jacobsson, S., Jamison, A. & Rothman, H. (1986). *The biotechnological challenge.* New York: Cambridge University Press.

Judson, H.F. (1979). *The eighth day of creation: Makers of the revolution in biology.* New York: Simon and Schuster.

Karp, L.E. (1976). *Genetic engineering: Threat or promise?* Chicago: Nelson Hall.

Kieffer, G.H. (1987). *Biotechnology, genetic engineering and society:* Monograph Series III. Reston, VA: National Association of Biology Teachers.

Koshland, D.E. (Ed.). (1986). *Biotechnology: The renewable frontier.* Washington, D.C.: AAAS Publications.

Marx, J. (Ed.). (1989). *A revolution in biotechnology.* New York: Cambridge University Press.

Mertens, T.R. (Ed). (1975). *Human genetics: Readings on the implications of genetic engineering.* New York: John Wiley & Sons.

North Carolina Biotechnology Center. (1989). *North Carolina companies in biotechnology, 1989.* Research Triangle Park, NC: Author.

Office of Technology Assessment - Congressional Board of the 98th Congress. (1987). *Commercial biotechnology: An international analysis.* Washington, D.C.: U.S. Government Printing Office. Order from The North Carolina Biotechnology Center, P.O. Box 13547, Research Triangle Park, NC 27709-3547.

Olson, S. (1986). *Biotechnology: An industry comes of age.* Washington, D.C.: National Academy Press.

Online International Limited. (1987). *The business of biotechnology: World biotech report.* New York: Online Publications.

Pines, M. (1984). *Inside the cell: The new frontier of medical science.* Washington, D.C.: U.S. Department of Health, Education and Welfare.

Pines, M. (1984). *The new human genetics: How gene splicing helps researchers fight inherited disease.* Washington, D.C.: Department of Health, Education and Welfare.

Prave, P., Faust, U., Sitting, W. & Sukatsch, D.A. (Eds.). (1987). *Basic biotechnology: A student's guide.* New York: VCH Publishers.

Rodriguez, R.L. & Tait, R.C. (1983). *Recombinant DNA techniques: An introduction.* California: Benjamin/Cummings Publishing Company.

Rosenfield, I., Ziff, E. & Van Loon, B. (1983). *DNA for beginners.* Great Britain: A Wheaton and Co. Ltd.

Teich, A.H., Levin, M.A. & Pace, J.H. (1985). *Biotechnology and the environment: risk and regulation.* Washington D.C.: AAAS Publications.

Vasil, I.K. (1987). *Biotechnology: Perspectives, policies and issues.* Florida: University of Florida Press.

Watson, J. D., Tooze, J. & Kurtz, D.T. (1983). *Recombinant DNA: A short course.* New York: Scientific American Books.

Watson, J.D. (1977). *Molecular biology of the gene* (3rd ed.). Reading, MA: W.A. Benjamin.

Watson, J.D. (1981). *The DNA Story: A documentary history of gene cloning.* San Francisco: W.H. Freeman and Company.

Wheelis, M. & Gonick, L. (1983). *The cartoon guide to genetics.* New York: Harper and Row.

Witt, S.C. (1985). *Briefbook: Biotechnology and genetic diversity.* San Francisco: California Agricultural Lands Project.

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